

**PULMONARY PERMEABILITY AND
INFLAMMATORY MEDIATORS IN SUBJECTS AT-
RISK OF AND WITH ESTABLISHED ARDS**

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GLOSSARY OF ABBREVIATIONS

| | |
|----------------------------|---|
| ACE | Angiotensin converting enzyme |
| ALI | Acute Lung Injury |
| ARDS | Adult Respiratory Distress Syndrome |
| ARSAC | Administration of Radioactive Substances Advisory Committee |
| BAL | Bronchoalveolar lavage |
| CT | Computed tomography |
| D(A-a)O₂ | Alveolar-arterial oxygen gradient |
| DAD | Diffuse alveolar damage |
| DTPA | Diethylenetriaminepenta-acetic acid |
| ELAM-1 | Endothelial-leucocyte adhesion molecule-1 |
| ELISA | Enzyme linked immunosorbent assay. |
| ET | Endothelin |
| FiO₂ | Fractional inspired oxygen concentration |
| HRCT | High resolution computed tomography |
| ⁶⁷Ga | Gallium-67 |
| ¹²⁵I | Iodine-125 |
| IL-8 | Interleukin-8 |
| ¹¹¹In | Indium-111 |
| ¹¹³In | Indium-113 |
| K_f | Microvascular membrane filtration coefficient |
| kDa | Kilodaltons |
| keV | Kiloelectron volts |
| LAP | Left atrial pressure |
| LIS | Lung injury score |
| L/H | Lung/Heart |
| LVEDP | Left ventricular end-diastolic pressure |
| MBq | Megabequerel |
| MoAb | Monoclonal antibody |
| MOF | Multiple organ failure |

| | |
|-------------------------|--|
| MODS | Multiple organ dysfunction syndrome |
| P_{mv} | Hydrostatic pressure in the microvessels |
| P_{pmv} | Hydrostatic pressure in the interstitium |
| PAI | Protein Accumulation Index |
| PAP | Pulmonary artery pressure |
| PAWP | Pulmonary artery wedge pressure |
| PaO₂ | Arterial oxygen tension |
| PC | Personnel computer |
| PEEP | Positive end-expiratory pressure |
| PET | Positron Emmison Tomography |
| PMA | Phorbol myrisate acetate |
| PMN | Polymorphonuclear neutrophil |
| PPA | Plasma protein accumulation |
| PTCER | Pulmonary transcapillary escape rate |
| Q_r | Net transvascular water flow |
| RIA | Radioimmunoassay |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SEM | Standard error of mean |
| SIRS | Systemic inflammatory response syndrome |
| sL-selectin | soluble L-selectin |
| sE-selectin | soluble E-selectin |
| sP-selectin | soluble P-selectin |
| SP-A | Surfactant protein-A |
| ^{99m}Tc | Technetium-99m (Sodium pertechnetate) |
| t_{1/2} | Half-life (check I did use this) |
| Tm | Thrombomodulin |
| TNF | Tumour necrosis factor |
| VIIIIR:Ag | Factor eight related antigen |
| vWf | von-Willenrand factor |
| vWf:Ag | von-Willenrand factor antigen |

| | |
|------------------|--|
| vWf:RiCoF | von-Willebrand factor ristocetin cofactor |
| π_{mv} | Oncotic pressure in the microvessels |
| π_{pmv} | Oncotic pressure in the interstitium |
| σ | Reflection coefficient (a measure of the resistance to solute flux) |

DECLARATION

I hereby confirm that the this thesis is entirely ~~my own work~~ and composition.

Date 28/5/11 Signature of Candidate

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ABSTRACT

Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) represent a spectrum of acute inflammatory microvasculature lung injury which occur in an unpredictable manner in predisposed individuals following a number of disparate insults which are often remote from the lung itself. It is currently thought that circulating factors precipitate a systemic inflammatory response resulting in a pan-endothelial injury which accounts for their frequent association with multiple organ dysfunction syndrome (MODS). Although the incidence of ARDS is low, therapeutic options are limited, care is mainly supportive and the prognosis is poor. Impact on this dreadful situation is most likely to arise from research focusing the events which belie the initiation and perpetuation of the pulmonary inflammatory response, thereby facilitating the development of novel mechanism based therapies which may be delivered at the earliest stages in the development of lung injury.

Such research has been hampered by the fact that currently used diagnostic criteria do not address the underlying pathophysiological events and are thus not only non-specific but are insufficiently sensitive to the earliest stages of evolving inflammatory injury. Studies based on these criteria may thus encompass a population of patients who do not have true ARDS, but also exclude patients who suffer lesser forms of lung injury which arise from similar mechanisms and whose study might reasonably provide important information on the resolution of pulmonary inflammation.

One of the earliest pathophysiological events in the pathogenesis of acute inflammatory lung injury is damage to the delicate pulmonary endothelium with a resultant breakdown in the alveolar-capillary barrier leading to flooding of the pulmonary interstitium and alveolar airspace with a protein rich fluid. Techniques have been developed which utilise externally situated gamma detectors to chart the transit of radiolabelled proteins from the intravascular space to the pulmonary interstitium which provide a non-invasive and dynamic measurement which is both sensitive to and specific for the detection of inflammatory pulmonary oedema. Although of recognised importance in the evaluation of patients at-risk of and with established ARDS, such techniques have not been widely adopted. Although the reasons have not been documented, this may reflect the expense involved in the purchase of the equipment and radioisotopes and the limited accessibility of such equipment in a busy Intensive Therapy Unit.

The aim of this thesis was to assess the applicability of a fully portable dual isotope system which utilises three miniature scintillation detectors in the recording of pulmonary microvascular permeability. Recordings were carried out in the Intensive Care and High Dependency setting from patients both with established ARDS and from a group of patients at-risk of ALI/ARDS. In patients at-risk of ARDS I examined the hypothesis that it is possible to identify a subgroup of patients in whom a subclinical inflammatory response will be associated with the emergence of pulmonary microvascular permeability and also whether the occurrence of

pulmonary permeability represents a forme fruste which fails to progress or whether it is a reliable indicator of impending severe lung injury. Furthermore, in both groups of patients I wished to investigate whether the occurrence of enhanced microvascular permeability may be correlated with key inflammatory markers which may support contentions for their role in the pathogenesis of lung injury and also provide a true or surrogate marker for the presence or absence of enhanced microvascular permeability.

INTRODUCTION

1.1 THE DEFINITION OF ARDS.

The acute respiratory distress syndrome (ARDS) is a catastrophic form of acute microvascular inflammatory injury which forms a central component of multiple organ failure. It was almost certainly first recognised in young men catastrophically wounded in conflict situations who developed acute severe respiratory distress following multiple trauma and burn injury (Brewer et al. 1946), but the first published description of ARDS has been attributed to Ashbaugh and colleagues (Ashbaugh et al. 1967) who described twelve patients with acute respiratory distress characterised by severe dyspnoea, tachypnoea, cyanosis refractory to the administration of oxygen, decreased compliance of the respiratory system and diffuse alveolar infiltrations on the chest radiograph. They reported pathological findings in seven patients which included atelectasis, vascular congestion and haemorrhage, severe pulmonary oedema and hyaline membrane formation. This original report included one child, but a more detailed account of the condition was provided by the same centre, almost four years later, in which the authors changed the nomenclature of the condition substituting 'acute' with 'adult' (Petty et al. 1971). This was later regretted by the first author and has been corrected in the latest definition of the syndrome (Bernard et al. 1994).

1.1.1 Clinical features of ARDS

ARDS is described in a wide variety of clinical settings comprising both direct and indirect pulmonary injuries (**Table 1.1**), and characteristically becomes clinically apparent following a latent period, usually between 12 - 24 hours, after the initiating event (Fowler et al 1983). The underlying pathological reaction is complex involving multiple cellular and humoral mediators but at a tissue level is characterised by inflammatory injury to the alveolar-capillary membrane with a resultant increase in permeability to protein and solutes. The complexity of the pathological reaction and the contribution of individual elements remain to be clearly elucidated. As a result attempts to define ARDS have concentrated on the major operational features of the disorder such as the degree of hypoxaemia, the appearance of the chest radiograph and the compliance of the respiratory system. The central pathophysiological event, increased pulmonary permeability, is generally inferred by exclusion of factors likely to attribute to cardiogenic oedema.

A major advance in our understanding of ARDS came in 1988 with the proposition of an expanded definition based on three critical distinguishing features of the disorder (Murray et al. 1988). The first incorporated epidemiological data which suggested that two major groups of patients could be identified: those who make a prompt recovery and generally have a favourable prognosis and those who develop a chronic fibrotic illness. The majority of the mortality occurred in the latter group (Montgomery et al. 1985). This led to the classification of patients suffering from ARDS as either 'Acute' or 'Chronic'. Furthermore, the second feature in the expanded

definition came from an appreciation that lung injury was not of equal magnitude in all patients. A 'lung injury score' was formulated and the term ARDS attributed to those patients with only the most severe form of lung injury. (**Table 1.2**) The third part of the expanded definition allowed recognition, if it were known, of the underlying cause. (**Table 1.3**)

The merits of the expanded definition included an acknowledgement of the phase and aetiological precipitant of the disease which clearly had prognostic implications for the patient. In addition, the use of the lung injury score facilitated a grading of the severity of lung injury. The $\text{PaO}_2/\text{FiO}_2$ ratio avoided arguments over the correct level at which hypoxaemia could be defined and the chest radiographic score represented some assessment of the presence and severity of oedema. Although the compliance of the respiratory system was not essential, it again provided information on the severity and course of the disease. The value of the pulmonary artery catheter in the clinical management of patients was unquestioned, but the investigators recognised that it was inappropriate to base the diagnosis of ARDS on the value obtained and thus excluded this measurement from the diagnosis. The reasons for this will be explained in section 1.8.4.

In 1992 the first meeting of a consensus conference of North American and European investigators further refined the definition, particularly focusing on the spectrum of severity of lung injury (**Table 1.4**) (Bernard et al. 1994). Recognising that a continuum of lung injury existed they proposed the diagnostic entity of 'Acute Lung

Injury' (ALI) which was of lesser severity but shared similar precipitants and clinical course to ARDS. They also clarified the confusion regarding the term adult and recommended that investigators revert to the term 'acute'. A further recommendation was that all patients with a $\text{PaO}_2/\text{FiO}_2$ of less than 200 in the absence of left heart failure and/or interstitial lung disease should be considered to have ARDS regardless of the extent of their radiographic abnormalities and without making any allowance for the effect of positive end expiratory pressure on the PaO_2 .

1.1.2 Problems with the Definition of ARDS

Although considerable advances have been made in our understanding of inflammatory lung injury, both ALI and ARDS remain descriptive terms based on clinical criteria which do not address the underlying pathophysiological events pertinent to the development of inflammatory lung injury. The diagnosis usually rests on the appearance of the chest radiograph and arterial blood gases but these are both non-specific reflecting merely deranged pulmonary function without alluding to the cause. Although attempts have been made to ascribe a degree of specificity to certain appearances of oedema seen on the chest radiograph, these are often deceptive when applied to the individual patient (discussed in section 1.8.3) and other abnormalities which appear, such as ground glass opacification and pulmonary consolidation are seen in many other conditions which may occur independently, or co-exist with ARDS (Joffe. 1974, Putman. 1992). Inclusion of the pulmonary artery wedge pressure may provide an indirect assessment of the contribution of hydrostatic factors

to the clinical picture, but interpretation of this data may be misleading (discussed in section 1.8.4). The chest radiograph is also known to be insensitive to the earliest stages of lung injury when it is frequently normal (Greene et al. 1987). Furthermore the chest radiograph provides only a static record with no information on the dynamics of oedema formation/resolution. Whilst the pathology of ARDS has been documented in a number of studies (Hill et al 1976, Tomasheski. 1990) the difficulties associated with routine diagnostic biopsy in these patients precludes attempts to obtain structural information such as the presence of diffuse alveolar damage (DAD). Thus the current definition of ARDS remains imprecise and non-specific.

1.1.3 The epidemiology of ARDS

Controversy surrounding the definition of ARDS has led to variable estimates of incidence ranging from 1.5-3.5 per 100 000 population (Villard et al. 1989) to 75 per 100 000 population (Ashbaugh. 1972). Most authors quote a figure of 5-7 per 100 000 of the population (Osborne and Meyer. 1996). The recognition that lesser forms of lung injury constitute a similar though less severe form of the same disease process will undoubtedly lead to the identification of a greater number of cases (Bernard et al. 1994). Indeed, with the appreciation that the currently available techniques fail to identify a large subpopulation of patients with inflammatory lung

injury, those patients with clinically apparent ARDS have been likened to the tip of the iceberg in which a much larger number of subclinical cases remain to be identified.

1.1.4 Therapy in ARDS

The past thirty years have witnessed a dramatic improvement in the reception and initiation of care of the critically ill, particularly due to improvements in ventilatory modalities and other aspects of supportive care within the Intensive Therapy Unit. Studies are beginning to report improvements in survival rates for patients who suffer ARDS, particularly younger individuals with trauma-induced ARDS (Sloane et al. 1992, Milberg et al. 1995) although average survival figures remain around 50 percent. Nevertheless, the care of such patients continues to represent a significant drain on the Intensive care budget and exert a considerable human burden on both the relatives and carers of patients of succumb. In addition, those patients whilst some patients may make a dramatic and full recovery many are left with substantial pulmonary morbidity (Sloane et al. 1992, Hert and Albert. 1994, McHugh et al. 1994,)

Successful therapy for patients with ARDS would be likely to reap substantial economic benefit in addition to relieving the suffering of patients and their relatives. Considerable effort has been directed towards this goal but unfortunately little real progress has been made. Pharmacological agents have been delivered by both the

systemic and inhaled routes. Large trials of non-steroidal anti-inflammatory drugs and corticosteroids have been disappointing although there may be a role for corticosteroids for those patients who progress to the later fibroproliferative phase of the disease (Bernard et al. 1987, Bone et al 1987, Luce et al. 1988, Bone et al. 1989, Meduri et al. 1994). Encouraging reports observed with inhaled nitric oxide, a potent pulmonary vasodilator (Rossaint et al. 1993) await the result of further randomised controlled trials. More recently the potential role of aerosolised surfactant therapy has been examined and regrettably found to be without benefit (Anzueto et al. 1996).

Improvements in supportive care have included the development of ventilatory strategies directed towards improved systemic oxygenation and limiting further pulmonary barotrauma to the already damaged lung. Such strategies include, inverse ratio ventilation, in which the inspiratory phase is extended at the expense of a shortened expiratory phase (Cole et al. 1984, Lain et al. 1989) and high frequency jet ventilation employing rapid (at least 60 breaths per minute) ventilatory bursts with a tidal volume approximating to that of the anatomical dead space (Carlson et al. 1983). The use of extracorporeal membrane oxygenation (ECMO) continues to be used in certain centres in the USA with apparent success but controlled studies have demonstrated little difference between patient treated with ECMO and the control group (Morris et al. 1994).

Although further improvements in supportive modalities are to be expected, a major challenge for physicians and intensivists is to understand the earliest mechanisms in

the disease process which lead to the initiation and perpetuation of the acute inflammatory response. This in turn should lead to improved and earlier recognition of impending lung injury and the development of novel mechanism based therapies which could potentially be delivered at an earlier time point when it may be more realistic to expect lung injury to be attenuated or aborted (Reid et al 1995 (b)).

Thus in the remainder of this introductory chapter I intend to concentrate on what is known about the pathogenesis of the ARDS, highlighting the role of the endothelium and the principle effector cell, the neutrophil.

1.2 THE PATHOGENESIS OF ACUTE LUNG INJURY

1.2.1 Overview

ARDS is a catastrophic form of acute inflammatory microvascular lung injury which evolves rapidly involving a complex network of inflammatory cells and mediators. The earliest pathological events are believed to occur within the pulmonary microvascular bed where inflammatory injury to the delicate pulmonary endothelial and epithelial surfaces results in the leakage of protein rich fluid from the intravascular space to the pulmonary interstitium and alveolar airspaces. This leads to the appearance of bilateral interstitial shadowing on the chest radiograph and severe impairment of gas exchange, often requiring the administration of high

concentrations of oxygen and mechanical ventilation. Although at this stage some individuals will recover, others progress rapidly to a chronic phase. Alveolar type I cells are lost and replaced with the proliferation of type II alveolar pneumocytes leading to thickening of the alveolar septum. The interstitium becomes infiltrated with mesenchymal, inflammatory and other cells including fibroblasts which proliferate and deposit new scar matrix proteins which obliterate the alveolar, alveolar ducts and the pulmonary interstitium (Zapol et al 1979, Fukuda et al. 1987). Alterations are also seen in the pulmonary vasculature including the obliteration of the capillaries and thrombosis contributing to pulmonary hypertension (Tomashefski et al. 1983, Bachofen et al. 1974, Fukuda et al. 1987). Such extensively injured lungs are prone to develop secondary infection and many individuals progress within a systemic inflammatory response to multiple organ failure (**Figure 1.1**).

1.2.2 The Endothelium in Acute Lung Injury

At its most simple level the endothelium provides a selectively permeable barrier between the pulmonary interstitium and the intravascular space which facilitates the efficient regulation of gas exchange by the alveolar units. Breakdown of the integrity of this barrier function leading to flooding of the alveolar airspace therefore has serious consequences for the efficient function of the lungs but the endothelium is also known to play a central role in the acute inflammatory response.

As early as 1889, Cohnheim realised the importance of the endothelium in the regulation of the inflammatory response. Observing that focal injurious or inflammatory stimuli produced a localised inflammatory response and that the microvasculature downstream of the stimulus was uninvolved, he speculated that “inflammation is the expression and consequence of a molecular alteration in the vessel walls (Cohnheim. 1889). The explosion in endothelial cell biology in recent years has begun to elucidate the mechanisms by which the endothelium participates and regulates the inflammatory response.

The quiescent endothelium provides an antithrombotic, non-adhesive surface. Inflammation reverses the endothelial cell phenotype to promote the coagulation cascade and express adhesion molecules involved in the recruitment of leucocytes and other inflammatory cells. Many of the inflammatory signals which prime and activate inflammatory cells also promote the upregulation of endothelial adhesion molecules. For example both interleukin-1 (IL-1) and tumour necrosis factor (TNF) mediate the upregulation of endothelial adhesion molecules involved in the earliest phases of recruitment of inflammatory cells to the lung (Mantovani et al 1992).

In addition to the inflammatory response, endothelial damage in its broadest sense is likely to affect the synthesis of endothelial cell components. As such they may be used as markers of severity of endothelial cell injury in ARDS as reviewed in section 1.4.

1.2.3 ARDS as a Pan-endothelial Injury

The observation that many cases of ARDS arise from indirect pulmonary insults such as pancreatitis or perforated viscus lead to the concept that a circulating humoral factor must be involved in the pathogenesis of the disease. Thus it was anticipated that a global microcirculatory injury may occur in which ARDS merely represents the pulmonary component. Support for this theory has come from several studies in which significant structural alterations (accompanied by accumulation of neutrophils) have been documented in multiple organs following experimental sepsis or acute lung injury.

In an animal model of acute lung injury induced by intravenous administration of phorbol myristate acetate (PMA) Mizer and colleagues demonstrated that significant structural alterations occur in non-pulmonary organs (Mizer et al. 1989). Moreover these non-pulmonary organ changes developed concomitantly with acute lung injury and were associated with marked increases in parenchymal neutrophil accumulation. St John and colleagues demonstrated that this neutrophil accumulation was associated with a functional counterpart by demonstrating significant alterations in intestinal microvascular permeability in several animal models of acute lung injury. (St John et al. 1991, St John et al. 1993) Human cadaver evidence from patients who succumb within hours from their injuries suggests that the lungs are not the only organs affected by an inflammatory process (Nuytinck et al. 1988). The concept of a global microcirculatory injury is also borne out by the clinical experience. ARDS

and multiple organ dysfunction syndrome (MODS) share similar risk factors and ARDS is often associated with or may lead to MODS (**Figure 1.1**) (Gill and Sibbald. 1996).

1.2.4 The lung is the major target of a pan-endothelial injury

If such a concept of a global microcirculatory injury is operative, the question as to why the lungs are so often the first and principle organ to be involved must be addressed. Several factors are likely to be contributory.

The pulmonary circulation contains a large endothelial surface area which, at any one time, is in close proximity to a resident pool of neutrophils. Even in health, a subpopulation, which may be up to 50 % of the circulating neutrophils, are temporarily sequestered within a 'marginated pool' in close apposition to the pulmonary capillaries and thus systemic mediators are likely to act first upon this resident pool. Furthermore the lungs receive the entire cardiac output such that any such mediator will pass through the pulmonary circulation in high concentration. In addition the delicate structure of the alveolar unit is such that minor changes may result in a marked impairment of function.

1.3 INFLAMMATORY CELLS IN ARDS

1.3.1 The Neutrophil

A large body of evidence has amassed to implicate the neutrophil as a key effector cell in the pathogenesis of ARDS. Histological specimens taken from patients in the earliest stages of the disease display an intense neutrophilic infiltrate (Holter et al. 1986). Similarly, bronchoalveolar lavage fluid demonstrates a preponderance of neutrophils (Lee et al. 1981) as well as the neutrophil secretory products, elastase (Rocker et al. 1989) and collagenase (Christner et al. 1985). Furthermore, BAL neutrophilia has been shown to correlate with markers of disease severity including the degree of pulmonary permeability to proteins and the lung injury score (Sinclair et al. 1994).

In patients at-risk of ARDS, inflammatory markers of neutrophil activation and recruitment show potential to facilitate the identification of those patients at highest risk of ARDS progression. Bronchoalveolar lavage (BAL) fluid from patients suffering either multiple trauma, perforated bowel or pancreatitis with elevated levels of the neutrophil chemotaxin, interleukin-8 (IL-8), are at significantly higher risk of progression to established ARDS than those who do not (Donnelly et al. 1993). Similarly low levels of circulating soluble L-selectin, a neutrophil adhesion marker involved in the early stages of neutrophil-endothelial adhesion, also provide predictive potential for ARDS progression (Donnelly et al. 1994).

1.3.2 Other inflammatory cells

Although at first sight the case for the neutrophil may appear conclusive, as ARDS has been reported in neutropenic patients (Braude et al. 1985), thus the neutrophil is unlikely to represent the sole effector cell. Whether these patients represent a separate subgroup of ARDS or whether small numbers of neutrophils in the pulmonary tissues are sufficient to provide the critical link in a pathogenic sequence is unknown (Haslett. 1992).

1.3.3 Recruitment of Inflammatory Cells to the Lung

In order for neutrophils and other inflammatory cells to induce lung injury, they must first be recruited from the vascular space and migrate into the pulmonary parenchyma. This multistage event is facilitated by both the biophysical properties of the neutrophil and the pulmonary circulation but also by the complex interplay between different groups of adhesion molecules present on the endothelial and inflammatory cell (MacNee and Selby 1993). The mean diameter of the neutrophil is 7-8 μm (Schmidt-Schonbein et al. 1985) and that of the pulmonary capillary 5.5 μm (Guntheroth et al. 1982), thus in order to transit through the pulmonary circulation the neutrophil must deform. Experimental models and human studies have demonstrated that decreased neutrophil deformability increases neutrophil accumulation in the capillaries (Downey and Worthen. 1988, Downey et al. 1990), and reduced transit time through the lung (Selby 1991).

The contribution of adhesion molecules to neutrophil recruitment has largely been elucidated in the systemic circulation, but is also likely to play a key role in the lung (**Figure 1.2**). It is presently thought that molecules of the Selectin family are responsible for the earliest stages of leucocyte-endothelial adhesion and those of the integrin family, the later 'tight' adhesive phase which is a prelude to neutrophil transmigration. The known selectin family currently comprises three structurally related cell surface molecules involved in neutrophil-endothelial adhesion. Their nomenclature relates to the cell type on which the selectins were first described (Bevilacqua et al. 1991). P-selectin was first identified on activated platelets (Hsu-Lin et al. 1984, McEver et al. 1984), but was later found to be expressed on activated endothelium (McEver et al. 1989). E-selectin was first identified as a cytokine inducible molecule on the endothelium which facilitated the adhesion of neutrophils (Bevilacqua et al. 1987). L-selectin was first identified as a molecule responsible for the homing of lymphocytes into lymph nodes (Gallatin et al. 1983) and later recognised to play a key role in the early stages of neutrophil-endothelial adhesion in response to inflammatory stimuli (Kishimoto et al. 1989). Evidence that constitutively expressed neutrophil L-selectin interacts with its corresponding ligand on the endothelium to facilitate neutrophil deceleration and 'rolling' was provided by the development of specific monoclonal antibodies which resulted in a significant inhibition of early neutrophil/endothelial adhesion and a consequent reduction in neutrophil transendothelial migration (Lawrence et al. 1991). Following this short term adhesion, specific up-regulation of neutrophil integrin adhesion molecules, e.g.

CD11b/18 leads to firmer adhesion, bringing the circulating neutrophil to a halt and facilitating neutrophil -endothelial transmigration (Smith et al. 1988). During the transition to "tight" adhesion L-selectin is cleaved by a metalloprotease (Bennet et al. 1996) and exists in the circulation in a soluble form (sL-selectin) which continues to exert biological activity by retaining the capacity to bind to activated endothelium (Schleiffenbaum et al. 1992).

The importance of the selectin family has been further reiterated by the development of animal models through gene targeting leading to so-called "knock-out" mice. Null mutants of all three selectins have been produced in mice which confirm the importance of the selectin family in the early inflammatory response (Frenette and Wagner. 1997) and are likely to provide valuable insight into the role of the selectins in the pathogenesis of several inflammatory disease states.

1.4 EVIDENCE FOR ENDOTHELIAL DAMAGE IN ARDS

A number of endothelial markers have been examined in the setting of established ARDS. These include angiotensin converting enzyme (ACE), endothelin-1 (ET-1), von-Willebrand factor antigen (vWf:Ag) and thrombomodulin (Tm).

1.4.1 Serum Angiotensin Converting Enzyme

Angiotensin converting enzyme (ACE), the enzyme responsible for the catabolism of angiotensin I to angiotensin II, is synthesised by endothelial cells and expressed on their luminal surface. The endothelium is the source of the activity of this enzyme in the blood (Stewart et al. 1981). Thus it was hypothesised that the activity of this enzyme in blood or BAL fluid could reflect pulmonary endothelial damage. Animal models of acute lung injury demonstrated early acute rises in the circulating level of serum ACE which, as lung injury progressed, decreased to levels below baseline (Nukiwa et al 1982, Hollinger et al 1980, Gorin et al. 1981). Human studies have also demonstrated a reduction in the specific activity of serum ACE but this is a non-specific finding being seen in other critically ill patients (Casey et al. 1981, Fourrier et al. 1983, Fourrier et al. 1985). Furthermore, blood concentrations of ACE in patients with acute lung injury did not reliably reflect disease severity or predict outcome (Fourrier F et al. 1985).

1.4.2 Endothelin-1 (ET-1)

Endothelin-1 is a potent vasoconstrictor peptide which is produced by the endothelium in response to ischaemia (Miyauchi et al. 1989), endotoxic shock (Morel et al 1989) and sepsis (Pittet et al. 1991). As it is predominantly metabolised by pulmonary endothelial cells it has been suggested as an indicator of acute lung injury (Sirvio et al. 1990). Elevated levels have been described in the setting of lung injury, and indeed seemed to reflect the underlying severity (Druml et al. 1993).

However ET-1 is non-specific with elevated levels also being described in pulmonary hypertension (Giaid et al. 1993) and asthma (Kraft et al. 1994).

1.4.3 Von-Willebrand Factor Antigen

The human plasma coagulation Factor VIII circulates as a complex of Factor VIII (VIII:C) and von Willebrand factor. Von Willebrand factor is a glycoprotein composed of multimers of Factor VIII related antigen (VIII:Ag) that are essential for platelet adhesion to damaged endothelium and platelet aggregation in the presence of ristocetin. Although small amounts of vWf are produced by megakaryocytes and platelets (Sporn et al. 1985), it is predominantly synthesised by the vascular endothelial cells (Bloom et al 1973). Increased circulating vWf have been found in numerous studies where damage to the endothelium is thought to have occurred (Blann. 1993). vWf may be assayed immunologically as von-Willebrand factor antigen (vWf:Ag) or functionally as ristocetin cofactor (vWf:RiCoF). A disproportionate rise in vWf:Ag to vWf:RiCoF has been shown to occur in respiratory failure and this is thought to indicate damage to the pulmonary endothelium (Grant et al. 1978, Carvalho et al. 1982)

Carvalho examined the level of vWf:Ag and vWf:RiCoF in 100 patients with acute lung injury, 29 critically ill patients without lung injury and 60 normal controls. They found that in normal and critically ill patients without evidence of lung injury

the levels of vWf:Ag and vWf:RiCoF were closely correlated but in patients with acute lung injury the levels of vWf:Ag were elevated disproportionately to the level of vWf:RiCoF and the ratio of vWf:Ag:vWf:RiCoF increased with increasing severity of lung damage (Carvalho AC et al. 1982). Further studies have confirmed the role of vWf:Ag as a sensitive marker of endothelial cell injury and activation (Hamilton et al. 1987, Ribes et al. 1987) but it should be noted that under some experimental conditions vWf:Ag may also be released by the action of vasopressin without concomitant vascular endothelial cell injury (Jones et al. 1992).

1.4.4 Thrombomodulin

Thrombomodulin (Tm) is a glycoprotein expressed on the surface of the endothelial cell and functions as a high affinity receptor for thrombin with powerful anticoagulant properties. When thrombin is bound to Tm, it activates protein C, triggering the activation of factors Va and VIIIa and impairing the formation of new thrombin. Furthermore Tm prevents the action of thrombin on fibrinogen, platelets and factor V and therefore clot formation.

Thrombomodulin is widely distributed on the endothelium of human arteries, veins, capillaries and lymphatics in all organs and tissues except the brain. The highest content of TM are found in the lung and human placenta (Maruyama et al. 1985). Elevated circulating levels of Tm have been described in a variety of disease states characterised by damage to the endothelium, including ARDS (Takano et al 1990,

MacGregor et al. 1997). Furthermore, the expression of Tm is known to be modulated by agents implicated in the pathogenesis of ARDS including TNF, IL-1, endotoxin (Dittman. 1991, Sawada et al. 1992) and recently primed activated neutrophils (MacGregor et al. 1997).

Thrombomodulin may be cleaved from endothelial cells and circulates in the plasma in soluble form (Ishii et al. 1985). The precise physiological role of this soluble form is still unclear however it appears that release of soluble forms of thrombomodulin into the circulation reflects endothelial damage and not endothelial activation (Ishii et al. 1991).

1.4.5 E-selectin

E-selectin or endothelial-leucocyte adhesion molecule-1 (ELAM-1) is a 115 kDa glycoprotein which is involved in the mediation of early endothelial-leucocyte adhesion. It appears to be an endothelial cell specific. It is not expressed on resting endothelium but is inducible after stimulation with various inflammatory mediators including endotoxin, IL-1 and TNF alpha (Wong et al. 1991, Montgomery et al. 1991). In vivo, a constitutive low level of E-selectin expression appears to play a role in normal leucocyte trafficking (Frenett et al 1996).

The importance of E-selectin in the mediation of neutrophil influx was demonstrated by Mulligan and colleagues in a rat model of acute lung injury induced by the

deposition of IgG immune complexes . This model, which displays similarities to clinical ARDS, is critically dependent upon the activation of neutrophils and their physical contact and adherence to the endothelium. Using this model Mulligan and colleagues demonstrated the administration of both a monoclonal antibody to E-selectin or sialylated oligosaccharides which block the ligand of E-selectin (also shared by P-selectin) significantly reduced the amount of neutrophil extravasation and vascular permeability (Mulligan et al 1991, Mulligan et al. 1993)

A shed, soluble form of E-selectin (sE-selectin) may be detected in the supernatant of activated endothelial cells (Piggott et al. 1992) and a low level is present in the plasma of normal individuals (Newman et al. 1993). Several investigators have documented elevated levels in critically ill individuals including those with sepsis (Newman et al. 1993), the systemic inflammatory response syndrome (Cowley et al. 1994), and ARDS (Moss et al. 1996).

1.4.6 P-selectin

P-selectin is a glycoprotein of molecular weight 140 kD, that is stored in the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells and is rapidly translocated to the surface of these cells when they are activated by a range of mediators including TNF, oxygen radicals and thrombin (Stenberg et al 1985, Geng et al. 1990, Patel et al. 1991). As for E-selectin, a soluble form of P-selectin may be

detected in the plasma (Dunlop et al. 1992) and elevated levels of sP-selectin have been demonstrated in the plasma in acute lung injury (Sakamaki et al. 1995).

In a similar manner to E-selectin, the significance of P-selectin in the interaction between neutrophils and the vascular endothelium has been demonstrated using a rat model of neutrophil mediated lung injury induced by cobra venom factor. Monoclonal antibodies to P-selectin or the carbohydrate ligand recognised by P-selectin, significantly ameliorated neutrophil accumulation, extravascular leakage and haemorrhage (Mulligan et al 1992, Mulligan et al 1993)

1.5 ENDOTHELIAL MARKERS EXAMINED WITH A PREDICTIVE ROLE FOR ARDS

Given that advances in the management of ARDS are likely to arise from an earlier recognition of evolving lung injury, interest has focused on predictive indices. At a pathological level the earliest events appear to arise in the pulmonary microvasculature and the appearance of increased microvascular permeability is presumed to reflect inflammatory damage to the delicate pulmonary endothelium. As a result several groups have explored the possible role of circulating markers of endothelium damage in the at-risk period.

1.5.1 von-Willebrand factor Antigen

The potential of vWf:Ag to predict progression to lung injury was evaluated in a prospective study involving a cohort of patients with non-pulmonary sepsis syndrome (Rubin et al. 1990). A plasma level greater than 450% over control values was 87% sensitive and 77% specific with a modest positive predictive value of 65% for identifying patients with non-pulmonary sepsis and a normal chest radiograph who subsequently developed acute lung injury although this rose to 80% when the elevated plasma vWf:Ag was combined with the failure of at least one organ.

VWf:Ag is however unable to reliably predict impending acute lung injury in more diverse groups of patients. In one series of 96 patients from multiple at-risk groups analysis of VWf:Ag failed to identify those who subsequently progressed to ARDS (Moss et al. 1995). In a further study of 21 patients with a multiplicity of risk factors for ARDS, despite a vWf:Ag of > 450% in 6 of these patients, none progressed to ARDS (Sabharwal et al. 1995).

1.5.2 Other endothelial markers

Circulating levels of E- and P-selectin were not found to be predictive of subsequent progression to ARDS in patients suffering from multiple trauma, pancreatitis or perforated bowel (Donnelly et al 1994). This finding was also observed in a study of nineteen patients with sepsis and thirty-six suffering multiple trauma (Moss et al. 1996). In addition, we have previously examined the role of Tm in a group of fifty-

eight patients at-risk of ARDS, from a cohort of patients comprising multiple trauma, pancreatitis and perforated viscus and found that it was unhelpful in assisting with the prediction of patients who subsequently progressed to ARDS (Reid et al 1995 a).

1.6 NEUTROPHIL MARKERS IN ARDS

1.6.1 Neutrophil elastase

The human neutrophils contains a large number of potentially histotoxic substances (Haslett C et al. 1989) of which neutrophil elastase has emerged as one of the prime candidates responsible for tissue injury. It has the ability to digest many of the components of the alveolar-capillary membrane (Janhoff et al. 1979, Smedley et al. 1986) and has been found in large quantities in bronchoalveolar lavage fluid taken from the lung of patients with established ARDS (Rocker et al. 1989). Elevated circulating levels of neutrophil elastase [which represent a marker of neutrophil degranulation (Jochum et al. 1984)] have been demonstrated in patients with established ARDS (Zheutlin et al. 1986) as have elevated serum and BAL levels of 7S collagen, a N-terminal peptide of type IV collagen cleaved by HNE (Kawamura et al. 1994, Konodoh et al. 1992).

1.7 NEUTROPHIL MARKERS IN PATIENTS AT-RISK OF ARDS

1.7.1 Neutrophil elastase

Work from Edinburgh has demonstrated that in patients with severe multiple trauma, the initial circulating elastase sampled during resuscitation in the Accident and Emergency Department, assists in the identification of a sub-group who subsequently progress to ARDS (Donnelly et al. 1995).

1.7.2 sL-selectin

Our group has previously demonstrated that patients who progress to ARDS have significantly lower levels of circulating sL-selectin compared to those who do not. Furthermore the level of sL-selectin is of prognostic significance, strongly correlating with semiquantitative indices of lung injury (time spent on a mechanical ventilator, the lowest PaO_2/FiO_2 ratio) and with subsequent mortality (Donnelly et al. 1994). This low level may reflect widespread endothelial activation and binding of the circulating receptor to its endothelial ligand. Thus, this circulating sL-selectin may reflect important events occurring at the alveolar-capillary interface implicating endothelial activation in the early ARDS disease process.

1.8 PERMEABILITY IN ARDS

1.8.1. The Alveolar-Capillary Barrier

The arrangement of the alveolar-capillary barrier facilitates effective gas exchange and fluid flux. It is composed of the pulmonary capillary endothelium, the alveolar epithelium and their respective basement membranes (**Fig 1.3**). The interstitial space between them is asymmetrically arranged around the capillaries such that on one side the space is obliterated and the barrier is less than $0.5\mu\text{m}$ (facilitating efficient gas-exchange), and on the other side the space is larger ($1\text{-}2\mu\text{m}$) and is thought to be the site of fluid flux between the capillary and interstitium. Under normal circumstances there is flow of both water and protein across the pulmonary endothelium into the interstitial space, which is drained by the lymphatic system, but the balance of hydrostatic and osmotic forces ensure that the alveolar-capillary barrier functions as a semipermeable membrane, preventing the filtration of water and solute from the blood and interstitial space into the alveoli. Pulmonary oedema only develops only when the maximal lymphatic drainage rate is exceeded. This is summarised by the Starling equation (Starling, 1896). The modern version of this is:

$$Q_f = K_f [(p_{mv} - P_{pmv}) - \sigma (\pi_{mv} - \pi_{pmv})]$$

Q_f = net transvascular water flow

K_f = microvascular membrane filtration coefficient

p_{mv} = hydrostatic pressure in the microvessels

P_{pmv} = hydrostatic pressure in the interstitium

π_{mv} = oncotic pressure in the microvessels

π_{pmv} = oncotic pressure in the interstitium

σ = reflection coefficient (a measure of the resistance to solute flux)

The law is analogous to Ohm's law which states that Transvascular flow = Conductance (K) x Driving Pressure. Applied to the alveolar-capillary barrier, the driving pressure represents the difference between net transvascular hydrostatic pressure ($P_{mv} - P_{pmv}$) and the net transvascular protein osmotic pressure $\sigma (\pi_{mv} - \pi_{pmv})$. Normally , a positive hydrostatic pressure gradient ($p_{mv}-P_{pmv}$) tends to move liquid from the circulation to the interstitial space and this force is opposed by an oncotic pressure gradient ($\pi_{mv} - \pi_{pmv}$) that promotes the movement of liquid back into the vascular compartment. The net starling forces favour movement of liquid into the pulmonary interstitium where it is drained by the lung lymphatics. In the presence of an intact alveolar-capillary barrier the lung is relatively resistant to the accumulation of alveolar oedema, even at elevated hydrostatic pressures (Staub et al. 1967 Taylor. 1981).

1.8.2 Pulmonary Microvascular Permeability and the Diagnosis of ARDS

In contrast to the normal situation described above, ARDS is characterised by damage to the delicate pulmonary endothelial and epithelial surfaces with the result that the protein reflection coefficient of the membrane (σ) falls to zero, permeability

to proteins and other solutes increases. The capillary filtration coefficient (K_f) is also increased, leading to a decrease in the opposing interstitial oncotic gradient. Fluid flux becomes increasingly dependent on the hydrostatic pressure gradient allowing lung oedema to develop even at low pulmonary microvascular pressures.

Despite the central role of increased pulmonary microvascular permeability in the pathogenesis of ARDS, diagnostic techniques aimed at the detection and quantification of enhanced lung leak have not been widely adopted, are only available at a limited number of centres where they remain research tools. Thus, for the most part, investigators continue to infer the presence of enhanced permeability by exclusion of factors suggestive of hydrostatic (cardiogenic) oedema. This may be performed on clinical grounds but is more often proposed on the basis of a low pulmonary capillary wedge pressure although some investigators have suggested that certain features on the chest radiograph may be sufficiently specific to enable differentiation of cardiogenic from non-cardiogenic oedema. Both techniques, however, have limitations which make them less than ideal on an individual basis.

1.8.3 The chest radiograph in increased permeability oedema

As an imaging system the chest radiograph has many advantages. It is widely available, portable, reproducible, relatively cheap, and provides regional imaging. Opinion remains divided as to whether the chest radiograph can reliably differentiate between cardiogenic and non-cardiogenic oedema. The radiographic signs

considered most discriminatory in one series included the pattern of blood flow, the distribution of the oedema and the width of the vascular pedicle (Milne et al. 1985). In 50% of cases of cardiogenic oedema blood flow appeared preferentially diverted to the upper lobe vessels (a so-called “inverted pattern”) which was only seen in 10% of patients with ARDS. A peripheral distribution of oedema was the commonest pattern observed in patients with ARDS but was rarely seen in patients with cardiogenic oedema. Based on their overall findings the authors claimed an overall accuracy of the chest radiograph of 86-89%. These findings are however at variance with other studies using similar scoring techniques. Though the finding of a patchy or peripheral distribution of oedema has been shown to be highly specific (87%) for ARDS, its sensitivity is below 50% making it an unreliable sign when applied to an individual patient (Aberle et al. 1988). In a similar manner the discriminatory value of further radiographic signs of interstitial fluid accumulation and pleural effusions have been questioned (Milne et al. 1985, Miniati et al. 1988, Smith et al 1987, Aberle et al. 1988).

1.8.4 The pulmonary artery catheter in increased permeability oedema

The introduction of a flexible, balloon tipped catheter into the pulmonary artery was first described by Jenkins and Bradley (Jenkins et al. 1970) but later popularised by Swan (Swan et al. 1970) who demonstrated that measurement of the pressure distal to an occluded balloon in the pulmonary artery (PAWP), correlated with the left

atrial pressure (LAP) and hence the left ventricular end-diastolic pressure (LVEDP) and thus had the potential to provide valuable haemodynamic and diagnostic information. The popularity of such catheters in clinical use is reflected by the fact that over two million are currently sold world-wide (Ginosar and Sprung. 1996). In the setting of ARDS, it was suggested that the finding of a low pulmonary artery wedge pressure infers any radiographic oedema seen is secondary to increased pulmonary permeability. However, for a variety of both technical and pathophysiological reasons, the PAWP may not always adequately reflect the LVEDP and may thus be misleading in individual patients (Raper and Sibbald. 1986). Furthermore, as the pulmonary artery catheter is often introduced several hours after presentation, data obtained may not reflect the initial haemodynamic profile of the patient (Dicpinigaitis PV. 1994). As mentioned, the expanded definition (Murray et al. 1988) excluded measurements made by the pulmonary artery catheter from the diagnostic algorithm.

Recently concern has been raised regarding the routine use of the pulmonary artery catheter following a prospective cohort study of 1789 patients with acute respiratory failure including ARDS and pneumonia was associated with an increased risk of death (Connors et al. 1996). A recent consensus conference on the use of the pulmonary artery catheter emphasised the ability of the correct use of the pulmonary artery catheter to assist in the diagnosis of patients with respiratory failure but recognised that the precise role of the catheter had yet to be defined (Pulmonary Artery Catheter Consensus Conference Participants. 1997).

Thus, the limitations of both the chest radiograph and the pulmonary artery wedge pressure emphasise the requirement for further research into methods specifically aimed at the detection of increased pulmonary microvascular permeability.

1.8.5 Techniques aimed at the Detection of Increased Pulmonary Microvascular Permeability

Techniques to investigate pulmonary permeability may be divided into two broad categories directed at either the epithelial or endothelial barrier. As the epithelial barrier is thought to be the rate limiting step in the pathogenesis of alveolar flooding, techniques which depend on sampling of alveolar or bronchial secretions most probably reflect both endothelial and epithelial permeability.

(A) Epithelial Permeability.

The most widely accepted method of measuring pulmonary epithelial permeability utilises a low molecular weight, water soluble solute diethylenetriamine pentaacetic acid (DTPA) radiolabelled with technetium-99m and delivered to the airways in the form of a aerosol. The rate of clearance from the lung is then recorded by either scintillation counters or a gamma camera. DTPA clearance is significantly accelerated in patients with ARDS, but although exquisitely sensitive, it is very non-specific (Hunter et al 1990).

(B) Endothelial permeability. The finding of high levels of protein in the oedema fluid of patients with ARDS led to speculation that the transit of radiolabelled proteins may assist in the quantification of pulmonary permeability. Anderson and colleagues demonstrated a highly significant increase in the clearance rate of radiolabelled albumin from patients with pulmonary oedema due to ARDS compared to that of left ventricular failure. However their technique involved the sequential sampling of tracheal aspirates which was time consuming and not necessarily representative of the alveolar secretions (Anderson et al. 1979).

This technique has now been superseded by non-invasive methods. The basis of these techniques was reported by Gorin (Gorin et al 1978). Working with a sheep model of inflammatory lung oedema induced by *Pseudomonas aeruginosa* bacteraemia, Gorin and colleagues used an externally situated gamma camera to record the interstitial accumulation of the plasma protein transferrin labelled with ^{113}In and found that it closely paralleled the concentration of protein measured directly in the lung lymph. The technique was subsequently extended for use in man (Gorin et al. 1980) and later simplified by Basran (Basran et al. 1985).

The principle of this technique depends on the assumption that the lung can be considered as a two-compartment model: an intravascular and extravascular compartment (**Figure 1.4**). A circulating plasma protein (commonly albumin or

transferrin) is labelled with a suitable radionuclide and the accumulation of this protein in the lung recorded by externally situated scintillation detectors. The count rates detected over the lung field provide a measure of both the intravascular marker and any which has accumulated in the pulmonary extravascular space. The simultaneous recording of count rates from the radiolabelled protein over the cardiac blood pool allow a correction for the normal clearance of the tracer from the circulation. Furthermore as changes in blood volume will alter the levels of recorded radioactivity detected by the probe(s), independent of changes in transvascular protein flux or plasma protein clearance, count rates from radiolabelled red blood cells are recorded in a similar manner. The ratio of the counts obtained over the lung to those over the heart may be plotted graphically as a component of time and the protein accumulation index mathematically derived (**Figure 1.5**). This is termed the plasma protein accumulation index (PPA) or protein accumulation index (PAI) and provides a dynamic non-invasive measurement of pulmonary microvascular permeability. A more complete explanation of this technique is given in section 2.5.1.

Applying a similar method to a dog model of inflammatory lung injury Dauber and colleagues demonstrated that the technique was both specific and sensitive, able to discriminate between hydrostatic pulmonary oedema and inflammatory pulmonary oedema caused by thiourea injury. They also demonstrated that it was possible to detect an almost doubling in pulmonary vascular protein leak prior to the appearance of any measurable increase in lung water (Dauber et al. 1985).

In man, the technique has been used to demonstrate a significant elevation of pulmonary protein accumulation in 22 patients with ARDS (11 with post-mortem evidence of diffuse alveolar damage) compared to 11 healthy volunteers (Rocker et al. 1988 a). In a further study, the technique was used in association with circulating and BAL markers of neutrophil activation to identify a graded intensity of pulmonary permeability in 50 patients with a range of respiratory failure and risk factors, supported the hypothesis that ARDS was not a distinct entity but rather a part of a spectrum of respiratory failure in which pulmonary permeability and neutrophil activation were related (Rocker et al. 1989). Further studies have shown that permeability to proteins is elevated throughout the course of ARDS and is also strongly related to the BAL neutrophil count and the lung injury score (Sinclair et al. 1994).

Other Techniques proposed to measure Pulmonary Permeability

PET Scanning

Positron emission tomography (PET) is based on the detection of the annihilation radiation. The technique utilises positrons, positively charged short-lived electrons, which readily combine with an electron to produce 2 gamma photons, each having an energy of 511 keV and are emitted in directions 180° opposed to one another. Although expensive, cumbersome and of limited availability, PET scanning is likely

to provide the most accurate data on pulmonary permeability (Schuster. 1989). The major advantage of PET scanning is the ability to obtain whole lung imaging and in addition, the simultaneous use of two or more positron emitting isotopes allows quantification of both protein leak and accumulation of extravascular lung water and pulmonary vascular blood flow in the same study.

Velazquez and colleagues performed studies on an animal model of lung injury induced by oleic acid. Using a PET scanner they demonstrated a strong correlation between the pulmonary transcapillary escape rate and the proportion of structurally abnormal alveoli as examined by both light and electron microscopy (Velazquez et al. 1991).

Calandrino and colleagues evaluated the pulmonary transcapillary escape rate (PTCER) of ^{68}Ga as an index of pulmonary microvascular permeability in 15 patients with ARDS and 12 healthy non-smoking controls. They found the PTCER to be up to 10 times greater than that of controls in the early phase of the disease, furthermore, repeat studies between 7 - 12 days after the onset of ARDS demonstrated reductions in the PTCER although it remained between 4 - 6 times greater than the controls (Calandrino et al. 1988).

Low molecular weight dextrans.

Abernathy and colleagues have investigated the feasibility of using radiolabelled dextrans. Using an in-situ rabbit lung preparations, they recorded the accumulation of 6 and 40 kDa dextrans, labelled with positron emitting isotope fluorine-18-fluoride (^{18}F) by means of a gamma camera. They found that the small dextrans traversed the microvascular membrane rapidly, leading to significantly reduced scan times which would have the potential advantage of minimising motion artefact (Abernathy et al 1995). This technique has yet to be employed in the clinical setting but has great potential since reduced scan times are likely to be significantly more user friendly to ITU staff and have the potential to reduce errors produced by the repositioning of probes in longer studies during which critically ill patients may require to be moved to facilitate nursing or paramedical staff.

1.9 PULMONARY PERMEABILITY AND THE PREDICTION OF ARDS

The first attempt to assess pulmonary microvascular permeability in a prospective manner was made in 1986 (Strum et al. 1986). Enrolling 22 patients with multiple trauma admitted to the Surgical Clinic, Hanover Medical School, West Germany, they prospectively evaluated lung capillary permeability by a radiolabelled albumin technique (Sugerman et al. 1984). The values obtained were compared with other physiologic parameters such as extravascular lung water (determined by the double indicator thermodilution technique), dynamic lung compliance, and alveolar-arterial oxygen tension gradients. Despite demonstrating variation between different

individuals and different time periods in the same individual the study was able to show that 87.5% of trauma patients demonstrated an abnormally elevated transvascular protein flux within 24 hours. In contrast, other physiologic parameters (including extravascular lung water) did not significantly deteriorate until 48-72 hours after trauma. The authors did not record progression to ARDS but this study represented an important attempt to provide documentary evidence for the hypothesis that the ARDS disease process was present prior to its clinical detection by other commonly used parameters. In 1988, Basran and colleagues demonstrated that an elevation in the protein accumulation index preceded the clinical appearance of ARDS in one patient with severe burn injuries and one patient with multiple trauma (Basran et al. 1988). In 1992, Braude and colleagues performed serial measurements of pulmonary microvascular permeability on a patient with cryptogenic fibrosing alveolitis who became febrile and breathless after an open lung biopsy and subsequently progressed to fulfil diagnostic criteria for ARDS. They demonstrated that an elevation in PAI preceded the radiographic deterioration and suggested that the use of pulmonary microvascular permeability measurement may predict the progression to ARDS.

Initial hopes that the measurement of protein accumulation might lead to earlier recognition of impending lung injury have not been realised by further studies which have tended to be small. In a study of ten patients undergoing cardiopulmonary bypass surgery the protein accumulation index (PAI), in the post-bypass period, was compared with other parameters of lung function. Whilst patients post-bypass

demonstrated an elevated PAI, this did not correlate with other parameters of post-operative lung function. Despite this the patient who generated a PAI of 3.2×10^{-3} quickly developed severe cardiorespiratory failure and died 3 days post-operatively (MacNaughton et al. 1992).

1.10 SUMMARY OF INTRODUCTORY CHAPTER

ARDS remains a dramatic disease and although uncommon, an important cause of untimely death. It is not as once thought, a distinct pulmonary entity, but represents the pulmonary component of a pan-endothelial injury, which in its most extreme, leads to multiple organ failure. Furthermore, we now recognise that within the context of the pulmonary component, a spectrum of lung injury exists with many more patients developing a less severe form of lung injury: ALI.

The pathogenesis of ARDS is complex: a multiplicity of mediators have been and continue to be described and the pathophysiological significance of many of these remains to be determined. The identification of individuals in the earliest stages of the disease process is likely to facilitate the identification of mediators critical to the initiation and perpetuation of inflammatory lung injury. A key early event, central to our understanding of ARDS is damage to the alveolar-capillary membrane resulting in leakage of fluid and protein from the circulation into the pulmonary interstitium

and alveolar airspaces. A number of techniques have been developed to record alterations in permeability but none have made the transition from bench to bedside. Consequently the recognition of ARDS continues to be based on the appearance of the chest radiograph and arterial blood gases and increased permeability is inferred by exclusion of factors which would otherwise point to cardiogenic oedema.

Aims of Study

Whilst significant progress has been made since the original description of ARDS in 1967, it is widely recognised that controversy continues to surround the diagnosis. In part, this reflects the multiplicity of disparate risk factors for ARDS, but largely is attributable to the fact that ARDS remains a clinical diagnosis and the criteria used do not directly reflect the underlying pathophysiological events. Central to our understanding of ARDS, and re-emphasised in the American-European Consensus statement definition, is the appearance of increased permeability to protein and solutes which results in flooding of the pulmonary interstitium and alveolar airspaces with proteinaceous fluid leading to non-compliant lungs, impaired oxygenation and diffuse radiographic appearances of oedema (Bernard et al 1994).. As yet a measure increased microvascular permeability is not available for routine use in the diagnosis of ARDS but in recognition of the importance and likely benefit of recording pulmonary permeability the investigators stated that “ because increased pulmonary permeability is considered a hallmark of ALI, research into methods quantifying altered permeability is encouraged.”

The aim of this thesis was to investigate a method of recording pulmonary microvascular permeability in patients both at-risk of, and with established ARDS and to correlate measurements obtained with inflammatory markers likely to reflect damage to the alveolar-capillary membrane. To this end, the evaluation of a fully portable double isotope system with miniature caesium-iodide scintillation detectors designed for ease of use in the ward and intensive care setting. The physical characteristics of the system with the appropriate radioisotopes are presented in a series of phantom experiments and bedside recordings obtained from normal volunteers, patients with established ARDS and a patient group at-risk of ARDS. In addition, the potential relationship between the recorded pulmonary microvascular permeability and a range of pathophysiological markers reflecting gas exchange, neutrophil activation and endothelial damage is investigated. Given the non-specificity of both serum ACE and ET-1, these markers were not examined in this thesis.

| Pulmonary | Extrapulmonary |
|---------------------|---------------------------|
| Pneumonia | Trauma |
| Aspiration | Sepsis |
| Pulmonary contusion | Perforated viscus |
| Smoke inhalation | Pancreatitis |
| Oxygen toxicity | Shock |
| Near Drowning | Cardiothoracic surgery |
| Pulmonary emboli | Burn injury |
| Radiation | Massive blood transfusion |
| | DIC |
| | Neurogenic |
| | Pregnancy |
| | Drug Overdose |

Table 1.1 Clinical Predispositions to Acute Lung Injury/Acute Respiratory Distress Syndrome. DIC = disseminated intravascular coagulation

| Component | Value |
|---|-------|
| 1. Chest radiograph | |
| No alveolar consolidation | 0 |
| Alveolar consolidation confined to one segments | 1 |
| Alveolar consolidation confined to two segments | 2 |
| Alveolar consolidation confined to three segments | 3 |
| Alveolar consolidation in all four segments | 4 |
| 2. Hypoxaemia Score | |
| $\text{PaO}_2/\text{FiO}_2 \geq 40$ | 0 |
| $\text{PaO}_2/\text{FiO}_2 - 30 - 39$ | 1 |
| $\text{PaO}_2/\text{FiO}_2 - 23 - 29$ | 2 |
| $\text{PaO}_2/\text{FiO}_2 - 13 - 22$ | 3 |
| $\text{PaO}_2/\text{FiO}_2 < 13$ | 4 |
| 3. PEEP Score (when ventilated) | 0 |
| PEEP - $\leq 5 \text{ cmH}_2\text{O}$ | 1 |
| PEEP - $6 - 8 \text{ cm H}_2\text{O}$ | 2 |
| PEEP - $12 - 14 \text{ cm H}_2\text{O}$ | 3 |
| PEEP - $\geq 15 \text{ cm H}_2\text{O}$ | 4 |
| 4. Respiratory System Compliance | 0 |
| Compliance - $\geq 80 \text{ ml/ cmH}_2\text{O}$ | 1 |
| Compliance - $60 - 79 \text{ ml/ cmH}_2\text{O}$ | 2 |
| Compliance - $40 - 59 \text{ ml/ cmH}_2\text{O}$ | 3 |
| Compliance - $20 - 39 \text{ ml/cmH}_2\text{O}$ | 4 |
| Compliance - $\leq 19 \text{ ml/ cmH}_2\text{O}$ | |

The final score is obtained by dividing the aggregate sum by the number of components that were used.

| Classification | Score |
|------------------|------------|
| No Lung Injury | 0 |
| Mild to Moderate | 0.1 - 2.25 |
| Severe | > 2.25 |

Table 1.2 Lung Injury Score proposed by Murray

| Part | Definition |
|------|--|
| 1. | Acute or Chronic, depending on course |
| 2. | Mild to moderate or severe lung injury depending on lung injury score |
| 3 | <p>Caused by (direct)</p> <p>aspiration pneumonitis</p> <p>fat embolism</p> <p>drugs</p> <p>toxic gas</p> <p>infections</p> <p>or associated with</p> <p>sepsis</p> <p>multiple blood transfusions</p> <p>acute pancreatitis</p> <p>disseminated intravascular coagulation</p> |

Table 1.3 Three part definition of parenchymal lung injury as proposed by Murray

| Timing | | Oxygenation | Chest Radiograph | Pulmonary Artery Wedge Pressure |
|---------------|-------------|---|---|--|
| ALI criteria | Acute Onset | $\text{PaO}_2/\text{FiO}_2 \leq 300$ mmHg (regardless of the level of PEEP) | Bilateral infiltrates seen on PA chest radiograph | ≤ 18 mmHg when measured or no clinical evidence of left atrial hypertension |
| ARDS criteria | Acute Onset | $\text{PaO}_2/\text{FiO}_2 \leq 200$ mmHg (regardless of the level of PEEP) | Bilateral infiltrates seen on PA chest radiograph | ≤ 18 mmHg when measured or no clinical evidence of left atrial hypertension |

Table 1.4 Recommended criteria for acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) proposed by the American-European Consensus Conference on ARDS 1994.

| | Cardiogenic oedema | Non-cardiogenic oedema |
|----------------------------|--------------------|------------------------|
| Heart size | Increased | Normal |
| Vascular pedicle | Normal/enlarged | Normal/reduced |
| Distribution of blood flow | Inverted | Normal/balanced |
| Septal lines | Not common | Absent |
| Peribronchial cuffing | Very common | Uncommon |
| Air bronchogram | Not common | Very common |
| Distribution of oedema | Even | Patchy and peripheral |
| Pleural effusions | Very common | Not common |

Table 1.5 Features on the plain chest radiograph which may assist in differentiating cardiogenic from non-cardiogenic pulmonary oedema

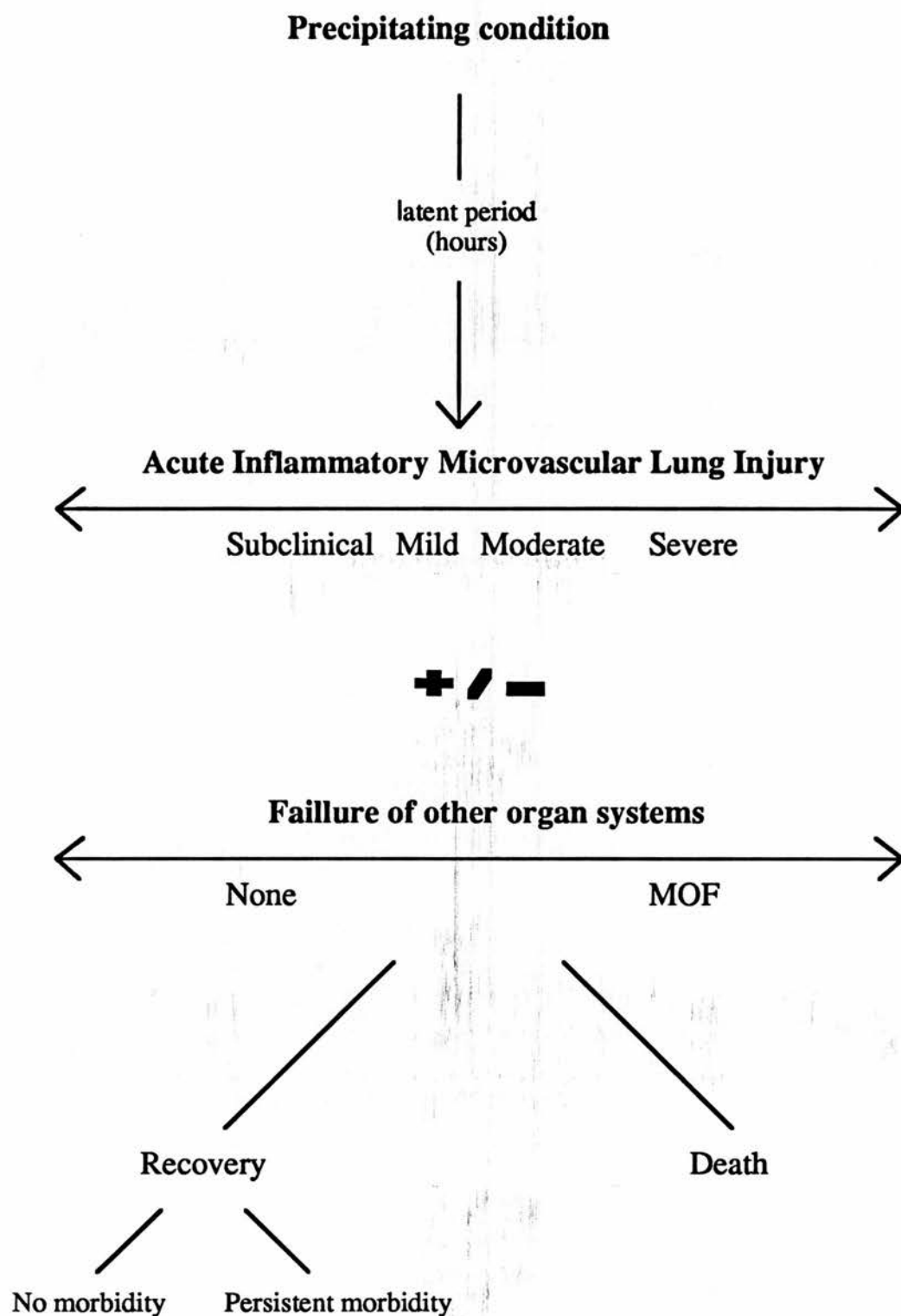


Figure 1.1 The spectrum of acute lung injury. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) develop in susceptible individuals, after a variable latent period, following a number of disparate precipitating conditions. ARDS and multiple organ failure (MOF) share common risk predispositions and may occur in the same individual. Mortality remains high and while some patients may make a spectacular recovery many are left with persisting pulmonary morbidity




| | Rolling Adhesion | Activation and Firm adhesion | Trans endothelial migration |
|-------------|---|--|--|
| Neutrophil | L-selectin  | L-selectin shed β -integrins  | β -integrins PECAM-1(CD31)  |
| Endothelium | E-selectin P-selectin | ICAM-1 ICAM-2 VCAM-1 Mad CAM | ICAM-1 PECAM-1(CD31) VCAM-1 |

Figure 1.2 Recruitment of inflammatory cells to the lung. The selectin family of adhesion molecules mediate low level adhesion causing the neutrophil to decelerate and roll along the endothelium. Thereafter upregulation of the integrin family of adhesion molecules leads to firm adhesion which is a prelude to transmigration.

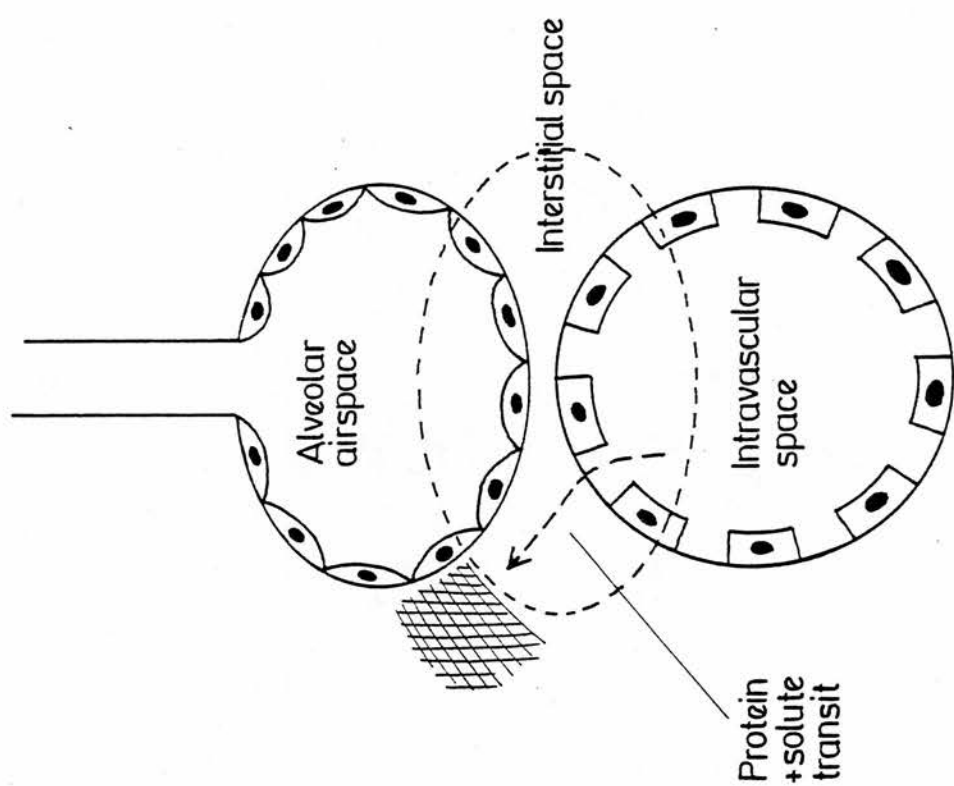


Figure 1.3 The alveolar-capillary membrane is composed of the alveolar epithelium, the capillary endothelium and their respective basement membranes, between which lies the interstitial space. Under normal circumstances there is flow of water, protein and other solutes from the intravascular space into the interstitial space which is then removed by the pulmonary lymphatic system.

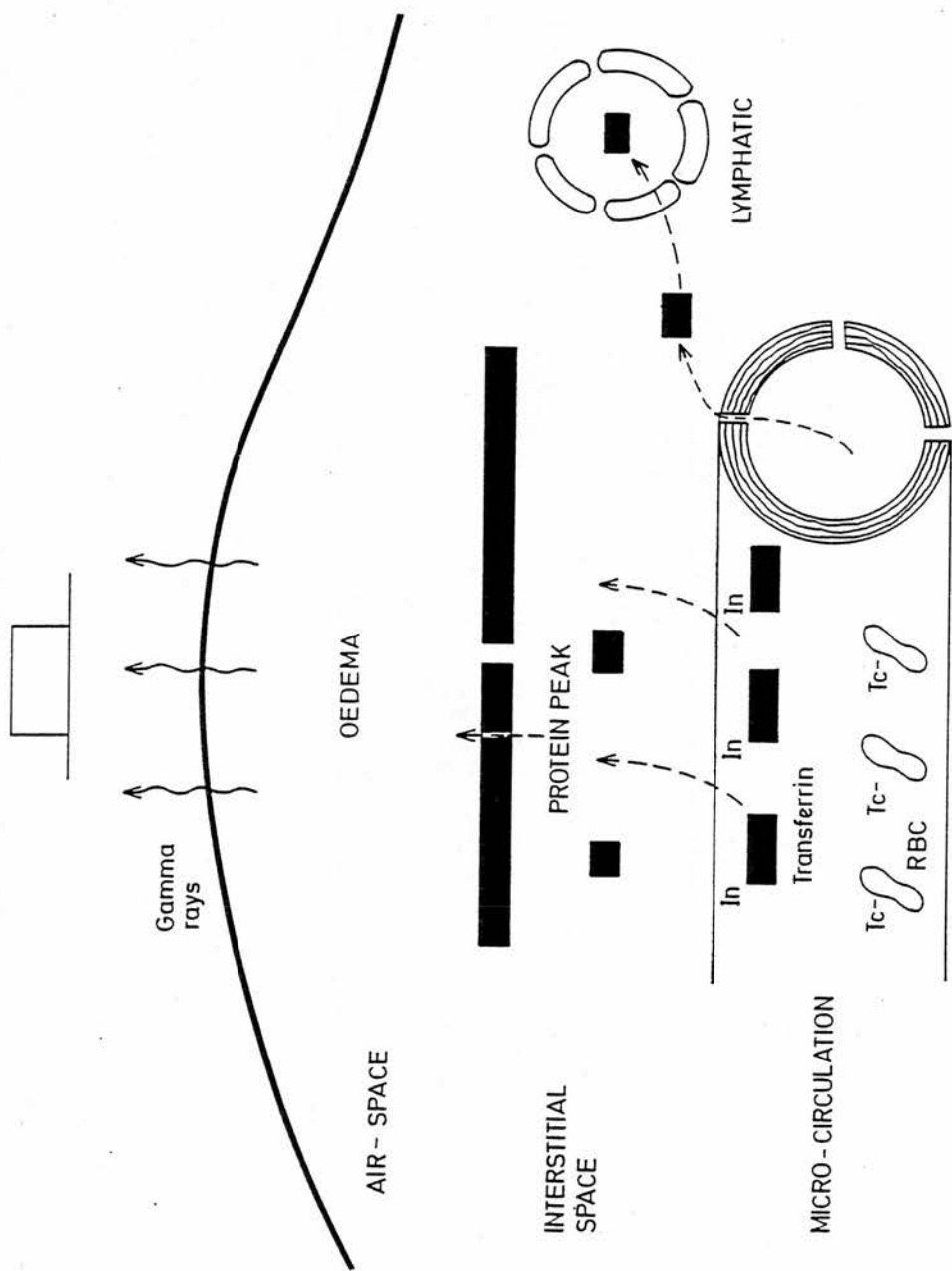


Figure 1.4 Theory of the non-invasive measurement of pulmonary endothelial permeability. The lung is considered to represent a two compartment system: an intravascular and an extravascular (interstitial space and alveolar airspace) compartment. The circulating plasma protein transferrin is labelled with a gamma emitter such as Indium (In) which allows its transit to be recorded in a non-invasive manner by a gamma detector positioned over the chest. When excessive leak occurs which exceeds the ability of the lymphatic system to remove the protein an accumulation occurs in the interstitial or alveolar airspace. Circulating erythrocytes are labelled with technetium (Tc) to facilitate corrections for circulating pulmonary blood volume.

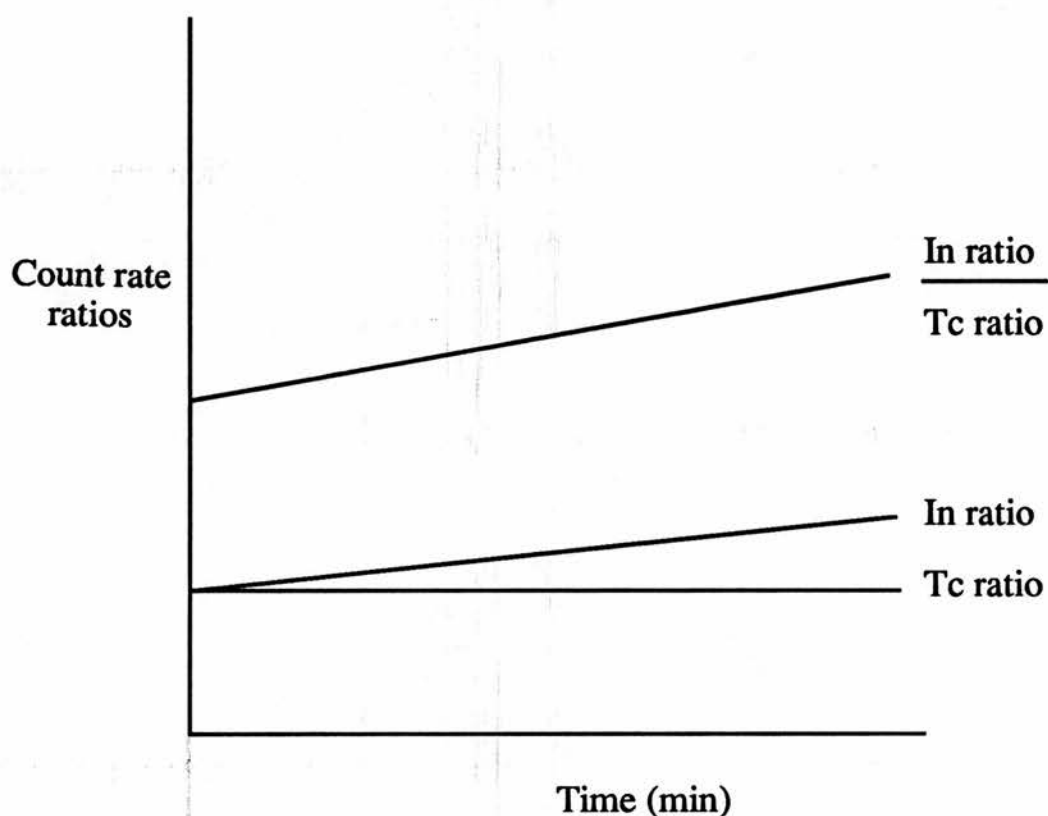


Figure 1.5 Representation of the graphs used in the calculation of the protein accumulation index. In this example the indium ratio (ratio of indium counts detected over the lung relative to those detected over the heart during the study period) has increased but the technetium ratio (lung/heart counts for Tc) has remained the same demonstrating that the intravascular volume did not change significantly during the study period. The final indium/technetium ratio demonstrates an extravascular accumulation of indium has occurred, such as would be expected in a patient with increased microvascular permeability.

METHODS

2.1 EQUIPMENT

For the purposes of these studies the Mediscint System (Oakfield Instruments Ltd, Oxford, U.K.) was chosen. This is an integrated system consisting of a personal computer (PC) based multi-channel analyser linked to three miniature scintillation counters (**Figure 2.1**). The system detects gamma radiation emitted by radio-labelled pharmaceutical within the patients body and is capable of detecting and counting from two radionuclides simultaneously. The mediscint software programme runs under the Microsoft Windows 3.1 operating system and provides real time display of acquired time activity curves. The data package facilitates analysis of the generated curves.

2.1.1 Scintillation Detectors

Scintillation detectors utilise the property of phosphors (commonly sodium or caesium iodide), which, when they absorb energy from ionising radiation, re-emit the energy in the form of light. Phosphors cannot detect beta emissions but are very sensitive for gamma emissions. The magnitude of the flash of light is converted to a digital signal, proportional to the amount of energy deposited in the scintillator by the radiation, and therefore provides a measure of the intensity. When connected to

appropriate electronic circuitry, the detector can perform a spectrum analysis of the energy of the radiation and distinguish between radiation's of different energies.

The Mediscint system utilises miniature scintillation detectors with caesium iodide crystal (**Figure 2.2**). The crystal is 14 x 14 mm in size and is housed within a lead collimator. Direct optical coupling between the crystal and large area photodiode eliminates the need for bulky photomultipliers and shielding thus giving a small lightweight detector which is easily attached to patients and is therefore relatively user friendly in both the ward and Intensive Care setting.

2.1.2 Computer

A Cardstar portable PC (Portable and Upgrades Ltd, Stockport, U.K.) was chosen in order to accommodate an IBM PC compatible half-size card providing eight channels of scaling and facilitate connection to the IBM bus.

2.2 RADIONUCLIDES

In these studies it was elected to use sodium pertechnetate (Technetium-99m) (Amersham International Code N0 INS IP) and Indium-111 (Amersham International Code N0 MCC 20). These radionuclides were chosen principally to facilitate the in vivo labelling of the circulating protein transferrin and erythrocytes.

2.2.1 Labelling circulating transferrin

As albumin is quantitatively the major plasma protein many investigators have chosen to label this protein. Early reports utilised albumin labelled with iodine-125 (^{125}I) (Anderson et al. 1979) however iodine-125 has a half-life of 59.6 days and as such would be unsuitable if repeated studies were to be contemplated. Much of the earlier work in the U.K has utilised indium-113 (^{113}In) which is no longer available. As an alternative to ^{113}In , both gallium-67 (^{67}Ga) and indium-111 (^{111}In) have been used to label circulating transferrin.

^{67}Ga is cyclotron produced, decays by electron capture and releases three different gamma photons: 93 keV in 38% of its disintegration's, 185 keV in 24% and 300 keV in 16 percent, the half-life is 3.2 days. ^{67}Ga has been used by European investigators in the determination of pulmonary microvascular permeability (Raijmakers et al. 1993, Raijmakers et al. 1995). However concerns have been raised regarding the efficiency of protein binding of ^{67}Ga . In a similar manner to ^{111}In , ^{67}Ga binds to the circulating plasma protein transferrin, although it does so less avidly than ^{111}In and may therefore dissociate from its protein bound state at a greater rate throughout the study period. The dissociated molecules of ^{67}Ga are then free to diffuse into the interstitium of the lung and bind to transferrin already present from leakage prior to the study period thus potentially giving rise to falsely high recordings (Raijmakers et al. 1992). In these studies we chose to employ ^{111}In .

Indium binds rapidly and efficiently with transferrin utilising the iron binding sites on this protein. ^{111}In chloride decays by electron capture and produces two energy peaks: 171 and 245 keV. It may also be administered intravenously without the requirement of withdrawing blood and handling blood samples. Disadvantages compared to ^{113}In include a half life of 2.8 days which results in a greater radiation dose to the patient and as ^{111}In is cyclotron produced, it is expensive and requires advance ordering and preparation. Furthermore indium is taken up by the bone marrow which would lead to high background readings on subsequent days and possibly interfere with the interpretation of repeated studies. Nevertheless, investigators in the Department of Nuclear Medicine and Intensive Care Unit, Queens Medical Centre, Nottingham had been using Indium-111 for the same purpose and had reported initial promising results (Alan Perkins, Department of Nuclear Medicine, Nottingham University, personal communication).

2.2.2 Labelling circulating erythrocytes

Technetium-99m ($^{99\text{m}}\text{Tc}$) has been used extensively as a label of erythrocytes. It decays by isometric transition emitting a 140.5 keV gamma photon in 88% of its disintegration's and has a half-life of 6.03 hours. It may be administered by intravenous injection and when preceded by stannous pyrophosphate (see below) approximately 40 - 50% of the injected dose is taken up by the skeleton within 3 hours. Up to 50% of the injected dose is cleared by urinary excretion within the first 3 - 6 hours. The activity retained on the erythrocytes remains unchanged from the

time of injection to about 4 hours post injection when a slight decrease can occur (Pavel et al. 1977)

Stannous Pyrophosphate (Pyroscint) Medgenix Code 2003002

Stannous pyrophosphate facilitates in vivo labelling of red blood cells by ^{99m}Tc by soaking them with stannous ions. It is administered intravenously at least 15 minutes prior to the administration of technetium-99m.

2.3 RADIATION SAFETY

I attended the University of Edinburgh Radiation Protection Course June 1994. All work involving the handling and administration of radioactive substances was carried out under the guidance of Dr Malcolm Merrick, Consultant in Nuclear Medicine, Dr Jim Hannon, Medical Physics Department, Western General Hospital and Dr Alistair Millar, Radiopharmacy, Edinburgh Royal Infirmary.

Radionuclides were drawn up in a designated and supervised area. When handling radioactive materials a laboratory coat, gloves and radiation film badge were worn. Working behind a lead-glass shield, radionuclides were drawn into syringes over a spill tray. The radioactive source was handled with tongs and the syringe containing the radiopharmaceutical transported in lead syringe holders. Radiopharmaceuticals were administered only by myself. The syringe and needle were disposed of in

dedicated indium and technetium bins located in the Radiopharmacy Department of the Edinburgh Royal Infirmary, the Nuclear Medicine Department, Western General Hospital Trust and in Room 7, Rayne Laboratory, Edinburgh City Hospital.

2.4 SUBJECTS USED IN STUDIES

2.4.1 Healthy Volunteers

Healthy volunteers were required by the Administration of Radioactive Substances Advisory Committee (ARSAC) to be over fifty years of age when studies involved the administration of the indium-111 radionuclide. Volunteers were recruited through an advertisement in a local newspaper requesting assistance from non-smoking persons over this age. Respondents were sent a written explanation of the project followed by a telephone call to ascertain if they wished to participate. Those who wished to continue with the study were invited to attend the Royal Victoria Chest Clinic, Chalmers Hospital and undergo a medical history, examination, chest radiograph and spirometry. Volunteers were given a further verbal explanation of the project and if agreeable to participate asked to sign the consent form. Following this selection process eight healthy male volunteers, median age 59 years (range 51-66) were enrolled. No patient had a history of recent or past respiratory illness and all were non-smokers. Chest radiograph and spirometry were within normal limits.

2.4.2 Patients with ARDS.

Patients with established ARDS were enrolled from the Intensive Care Unit at the Western General Hospital Trust, Edinburgh, Scotland. ARDS was defined using the American-European Consensus Statement (Bernard et al. 1994).

The lung injury score described by Murray and colleagues was used (Murray et al. 1988). The score is based on the systemic oxygenation, quantified as the $\text{PaO}_2/\text{FiO}_2$ ratio, and the appearance of the chest radiograph, the level of PEEP and when measured the respiratory system compliance. Using this system, lung injury may be quantified as mild to moderate (0.1 to < 2.5) or severe (≥ 2.5).

2.4.3 Patients at-risk of Acute Lung Injury and ARDS

A key element to this thesis was the study of patients who were predictably at-risk of ARDS. Previous studies in Edinburgh recruited patients with multiple trauma, perforated viscus and severe pancreatitis with a high rate of progression to ARDS (Donnelly et al. 1993). However, the unpredictable nature of their presentation presented logistic and technical problems for studies involving the use of indium-111 (^{111}In). As mentioned, ^{111}In is cyclotron produced and requires both advance ordering and preparation by the radiopharmacy department, it is also expensive thus it was likely that unless we enrolled patients from a predictable population, we

would undoubtedly waste considerable quantities of ^{111}In . I therefore chose to study patients undergoing oesophageal resection of malignant disease. During this procedure one lung undergoes deflation to allow access to the oesophagus and is thereafter handled during the operation and at the end of surgery reinflated. Rapid re-expansion is known to predispose to the development of pulmonary oedema (Pavlin et al. 1981, Henderson et al. 1985). The contralateral lung therefore receives the total ventilation and may be subject to barotrauma, also known to cause lung injury (Gammon et al 1992). This model has been utilised in similar studies of pulmonary permeability (Rocker et al. 1988 b). Patients undergoing oesophagogastrectomy were enrolled from the Cardiothoracic Unit, City Hospital, Edinburgh and The Western General Hospital Trust.

2.4.4 Ethics

All studies were approved by the Ethics Committee of the Lothian Health Board, Scotland, (reference numbers 1702/94/1/18, 1702/94/1/20, 1702/94/3/21), the management of each of the participating Hospital Trusts. Approval was also sought from the Administration of Radioactive Substances Advisory Committee (ARSAC) and the certificates were held by Dr Malcolm Merrick (reference numbers RPC 76-6 (62), RPC 76-6 (61) and RPC 162-26 (9)). Informed consent was obtained from each subject, or in the case of a ventilated subject, the next of kin.

2.5 STUDY PROCEDURES

2.5.1 Pulmonary Microvascular Permeability

(a) Protocol used for data acquisition.

Background counts were collected using 3 one minute counting periods. The Compton scatter protocol utilised 4 one minute counting periods and the study protocol utilised 180, 18 second counting periods to run for a total time of 54 minutes.

(b) Method of data collection

The Mediscint system was switched on 30 minutes prior to the study, at the end of this time, background counts are collected (the protocol used is stated above). Stannous pyrophosphate (0.4 mg/kg) was reconstituted in 10 ml of sodium chloride and two 10 ml saline flushes were drawn up. 10 MBq of indium-111 chloride was drawn-up into a syringe and the activity checked using a radionuclide calibrator (Capintec CRC-15R Radionuclide calibrator, Ramsay, New Jersey, USA). The syringe was then placed in a lead syringe holder before transport to the patient.

Following the collection of background counts, the reconstituted stannous pyrophosphate injection was administered by slow intravenous injection directly into

a large forearm vein (Millar AM et al. 1983). A butterfly needle was then sighted in a large forearm vein and 10 MBq ^{111}In administered, followed by a 10 ml saline flush. The right lung probe was positioned over the mid-zone in the mid-clavicular line and the left lung probe over the second interspace in the midclavicular line. The heart probe was placed over the left ventricle (position of maximum count rate). The probes were held in position by Mepore (Molnlycke, Sweden) and the Compton Scatter protocol commenced (see above).

Following the Compton Scatter protocol 10MBq of 99m-sodium pertechnetate was drawn into a syringe and the activity checked using a radionuclide calibrator (Capintec CRC-15R Radionuclide calibrator, Ramsay, New Jersey, USA). The syringe was transported to the patient in a lead syringe holder. No less than fifteen minutes after the pyrophosphate injection and following the completion of the Compton Scatter protocol, 10 MBq 99m-sodium pertechnetate was administered by slow intravenous infusion followed by 10 ml of normal saline flush. A further ten minutes were allowed for red cell labelling and mixing and the study protocol commenced. The study was terminated after the data collection period was completed.

Calculation of PAI from stored raw data.

The computer software stored the average count rate in counts per second (cps) recorded over the count period specified in the protocol for each one of the six

channels (2 channels per detector) simultaneously. Subsequent mathematical manipulations could be carried out on the results from each counting period for each detector.

Background correction:

Background counts were averaged for the three one minute counting periods recorded at the start of the study. If the background count rate was greater than one count per second the value recorded for that detector channel was subtracted from each stored value for that detector before further data manipulation.

Derivation of Compton downscatter correction factor:

This facilitated a correction for the number of counts due to ^{111}In which appear in the technetium window and would otherwise falsely elevate the apparent $^{99\text{m}}\text{Tc}$ count rate. Fifteen minutes after the injection of ^{111}In count rates were recorded in both the indium and technetium channels. The indium count which is measured in the technetium window is divided by count rate recorded simultaneously in the indium window. Four one minute counting periods were used and the average value obtained over these counting periods was used to derive the scatter correction factor for each detector.

Correction of the $^{99\text{m}}\text{Tc}$ count data for Compton scatter during the study:

The ^{111}In count rate for each period of the study was multiplied by the Compton correction factor derived above and the value subtracted from the simultaneously collected counts from each period in the $^{99\text{m}}\text{Tc}$ channel thereby deriving the corrected $^{99\text{m}}\text{Tc}$ count rate.

Derivation of the ^{111}In and $^{99\text{m}}\text{Tc}$ Lung/Heart (L/H) ratios:

For indium, the L/H ratio is obtained by dividing the counts recorded by the ^{111}In channel from the right and left lungs respectively by those counts simultaneously recorded by the ^{111}In channel over the heart. Using the corrected count rates for technetium the $^{99\text{m}}\text{Tc}$ is calculated in a similar manner.

Derivation of the extravascular ^{111}In counts:

The ^{111}In L/H ratio was divided by the $^{99\text{m}}\text{Tc}$ L/H ratio for each study period.

Relationship of extravascular ^{111}In counts to time and derivation of the PAI:

Each of the values derived above was plotted against time from the start of the data collection.

The computer is used to plot a line of best fit to the above plot using the least squares method and it is the slope of this line which is taken as the PAI (units of 10^{-3} per sec). The result is then multiplied by sixty to convert the result to minutes. When both lungs were studied the results from both lungs were averaged and recorded as the protein accumulation index (PAI) with units $10^{-3}/\text{min}$. A schematic representation of this was illustrated in Figure 1.5.

2.5.2 Blood Sampling

Venous blood:

Venous blood was withdrawn from a peripheral vein or when available, a central venous catheter. After sampling, blood was stored on ice until transport to the laboratory. Blood samples were centrifuged at 1000g x 10 minutes x 4°C, the supernatant then aspirated and stored at -70°C.

Arterial blood:

Arterial blood was withdrawn from an indwelling arterial line into a heparinised syringe and analysed immediately. Oxygenation was determined as the FiO_2/PaO_2 ratio derived from the circulating oxygen tension (kPa) and the inspired oxygen fraction ($\% \cdot 10^{-2}$).

2.5.3 Enzyme Linked Immunosorbent Assays

Soluble L-selectin

The sL-selectin ELISA was performed as described (Spertini et al. 1992). Briefly 96 well microtitre plates (Costar, Cambridge, MA) were coated with anti-L-selectin mAb (LAM 1.5 at 3µg/ml) and incubated overnight at 4°C. The wells were blocked with 2% bovine serum albumin and 1% gelatin for 1 hour at 37°C and then washed

with 0.1% Tween 20. Diluted patient serum was added (each sample is tested in triplicate). Each assay included the titration of a previously quantified standard plasma sample that was used to generate a standard dilution curve. After washing the plates were incubated with biotinylated anti-LAM 3 MoAb (1 μ g/ml) for one hour at room temperature. The plates were then washed and developed with o-phenylenediamine (0.125% w/v, Sigma) as a substrate in 0.1 M citrate buffer, pH 4.5 in the presence of 0.015% H₂O₂. The optical density (OD) of the reaction mix was quantified using a Dynatech MR5000 plate reader. Background OD values were obtained using wells albumin coated. The sensitivity of the ELISA was greater than 5 ng/ml.

von Willebrand ELISA

Circulating von-Willebrand factor antigen (vWf:Ag) was assayed by a double antibody sandwich ELISA according to previously described methods (Bartlett et al. 1976). Ninety-six well plates (Costar, Cambridge, MA) were coated with rabbit anti-human von-Willebrand factor (DAKO code A 082) at 5.4 mg/ml and incubated overnight at room temperature. Unabsorbed solution was discarded and the plates washed. Dilutions of the control (NIBSAC 5th British Standard) and samples were applied in triplicate and the plates incubated for two hours at room temperature following which time, peroxidase-conjugated rabbit anti-human von-Willebrand factor was added (DAKO code P226-HRP) and plates again incubated for two hours at room temperature. The plates were developed with tetramethyl-benzidine

Microwell Peroxidase Substrate (DYNATECH), the reaction stopped with 0.18 M sulphuric acid and the developed plates read on a Dynatech MR5000 plate reader at 450 nm.

Elastase Radioimmunoassay

Human neutrophil elastase was measured by a specific radioimmunoassay (RIA) with rabbit polyclonal antiserum according to the method (Bell et al. 1990). The antigen was purified from human neutrophils after leucopheresis (Calbiochem, Nottingham, UK). The antibody was specific for neutrophil elastase and did not cross react with pancreatic elastase (Sigma) or neutrophil-free lysed human platelet concentrate. The RIA used a 50µl standard/sample added to 50µl of ¹²⁵I-labelled elastase (10ng/ml) and 50µl anti-elastase antibody diluted to 1:3000. After overnight incubation at room temperature, donkey anti-rabbit immunoglobulin was added. After shaking for 45 minutes at room temperature, bound complex was separated from free complex by sedimentation through 10% sucrose at 1g, aspirated and counted in a NE 1600 gamma counter (Nuclear Enterprises, Edinburgh, UK). The results were expressed as nanograms per millilitre and the intra-assay coefficient was < 5%.

Thrombomodulin ELISA

A commercially available ELISA kit was employed (Diagnostica Stago, via Shield Diagnostics, Dundee, Tayside, U.K.). The epitopes on T_m recognised by these antibodies have been described (Amiral et al. 1994).

ELISA's for E- and P-selectin.

The above were analysed using commercially available double-antibody sandwich ELISA kits (Bender Medical Systems, Vienna, Austria)

Calculation of ELISA Results

ELISA results were analysed with the Assay Zap software programme for Macintosh computer (Biosoft Cambridge, UK) which uses a weighted four parameter curve fit on log transformed data.. Sample concentrations were calculated by interpolation of their mean absorbance on the standard curve.

2.6 STATISTICAL METHODS

Statistical analysis was performed on Macintosh computer using Abacus concepts Statview II statistical package (Abacus Concepts, Inc., Berkeley, CA, 1987). Data are expressed in the text as mean (SD). Non-parametric statistical analysis was applied. Comparison between groups were made by Kruskal-wallis test and Mann-

Whitney U test. Spearman rank was used to perform correlation's between PAI and circulating mediators. Bonforoni correction was applied to correct for multiple ties. The paired Students t-test was used to compare the PAI between the deflated and ventilated lungs. A p value < 0.05 was accepted as statistically significant.



Figure 2.1 The portable Mediscint system (Oakfield Instruments LTD, Oxford, U.K.). Comprises an integrated system consisting of a Cardstar portable computer (Portable and Upgrades Ltd, Stockport, U.K.) linked to miniature scintillation counters (not shown in this photograph) through a multichannel analyser.



Figure 2.2 The miniature scintillation counter (Oakfield Instruments LTD, Oxford, U.K.) is a small, lightweight gamma detector which may be easily attached to the chest wall.

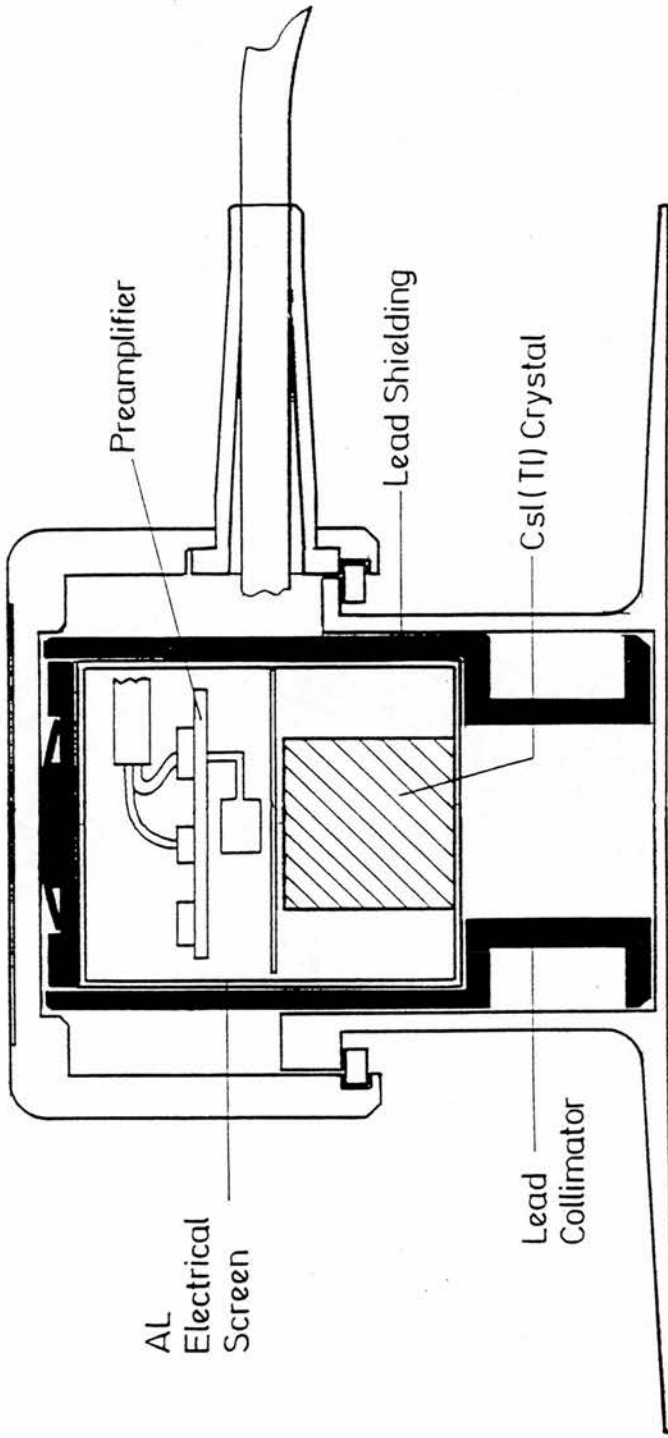


Figure 2.3 The miniature scintillation detector in cross-section. A small caesium-iodide scintillation crystal is housed within a lead collimator and is coupled directly to a large area photodiode.

EQUIPMENT

3.1 PERFORMANCE CHARACTERISTICS

3.1.1 Window settings

The system was set-up with a lower energy window of 88 - 170 keV (to include the photopeak and Compton scatter from ^{99m}Tc) and an upper window of 170 - 500 keV (to include both photopeaks of ^{111}In and as much possible of the Compton scattered radiation) (**Figure 3.1**). These settings were chosen to maximise sensitivity to ^{111}In , minimise positional sensitivity and facilitate the use of lower administered activities of the radionuclide to reduce the radiation dose to the patient. Although there was a cross-over from indium into the technetium windows it was possible to correct for this when analysing the study data (see section 2.5.1) As it would not be possible to correct cross-over of ^{99m}Tc into the upper window during studies sequential count rates were recorded in both the technetium and indium windows from point source of ^{99m}Tc .

Method

The experiments were performed in a dedicated room in the Radiopharmacy Department of Edinburgh Royal Infirmary. The Mediscint system was switched on

and allowed to run for approximately 30 minutes prior to commencing the experiment. A fixed point source of ^{99m}Tc was placed in a water bath and the distance from a miniature scintillation detector fixed to the external wall adjusted to record similar count rates observed in patients. Simultaneous recordings were made in both the technetium and indium windows.

Results and conclusion

The results are displayed in Table 3.1. It can be seen that the average cross-over of ^{99m}Tc into the upper window was less than one percent of detected counts. (mean = 0.73 %)

3.1.2 Dead Time

When a gamma ray strikes the scintillation material, the energy is transformed into a flash of light. During the period in which the scintillation crystal is processing the detected event, the equipment becomes refractory and consequently any gamma rays which strike the crystal during this period will not be registered. The duration during which a new event cannot be registered is termed dead time and is dependent upon the crystal and the electronics of the machine. If the administered activity of radionuclide gives rise to count rates which saturate the system significant dead-time losses may occur which would lead to an underestimate of the true count rate. In

order to ensure that the count rates detected in the patient groups would not be influenced by dead-time losses, the dead time was assessed as follows:

Method

The experiment was set-up as previously described in section 3.1. A fixed point source of ^{99m}Tc , at a sufficient activity to saturate the system, was placed in a perspex water-bath and a scintillation counter fixed in apposition to the external wall. The technetium was then allowed to decay over a 24 hour period and the activity recorded by the Mediscint system.

Results and conclusion

Figure 3.2 displays the count rate plotted against time. As count rates in the higher part of the curve are more likely to be subject to dead time losses, count rates from the lower part of the curve are used to calculate the dead-time of the equipment. I arbitrarily selected to use count rates obtained from 18 hours onwards and plotted the natural log of the count rate against time as shown in **Figure 3.3**. The extrapolation of this line to the y axis gives the line of least squares fit to the data and represents the natural log of the count rates expected to occur for the point source of ^{99m}Tc when no dead time losses are incurred. Taking the reciprocal of the natural log of the expected count rates allows calculation of the actual expected count rate and this is plotted, together with the observed count rates in **Figure 3.4**. It can be seen that at

higher count rates the difference between the observed and expected count rate increases reflecting greater dead time loss. The ratio of expected to observed counts allows calculation of the correction factor required at any given count rate and this is represented graphically in **Figure 3.5**. It can be seen that provided count rates are kept below 10 000 counts/sec, no correction is required for dead time losses with the Mediscint system.

3.1.3 Electrical Drift

The physical performance of a detector system may vary with the operating temperature (“electrical drift”). To ensure that no significant electrical drift would occur during the study period the following experiment was performed.

Method

The experiment was again set-up as described in section 3.1. A fixed point source of ^{99m}Tc (adjusted to avoid dead-time losses) was placed in a water bath at room temperature. Counts were recorded over a two hour period.

Results and conclusion

Count rates from ^{99m}Tc were corrected for decay and the corrected count rates transformed to their natural log and plotted against time as shown in **Figure 3. 6**. It can be seen that over a 1 hour study period, no significant electrical drift occurs and thus is unlikely to be a factor in influencing results obtained from the system during our study period.

3.1.4 Detector sensitivity as a function of position

To assess the relative sensitivity of the detector as a function of position within the patient, a series of phantom experiments were performed in a water bath. The activity of each source was adjusted to give a count rate below 5000 c/s and counts for ^{99m}Tc were corrected for anticipated decay assuming a half-life of 6.03 hours.

(a) Angle and Depth

The scintillation detector was fixed in apposition to the external wall of the perspex water bath, a point source of each radionuclide placed at increasing distances and angles from the centre of the detector and the count rate recorded.

Results and conclusion

Figure 3.7 demonstrates that for a point source of ^{111}In , the detected count rate falls rapidly when the source is positioned at an angle of greater than 30° from the centre of the miniature scintillation detector, suggesting that the volume of lung tissue sampled will lie within this narrow cone. *In vivo*, systemic distribution of the isotope will result in a broad beam rather than a point source rendering such an effect less critical. In a similar manner, it can be seen from **Figure 3.8 and 3.9** that as the scintillation counter is unfocused, the detected count rate to a point source is governed by the inverse square law and thus the detected count rate will be influenced by the thickness of the underlying chest wall. Furthermore, **Figure 3.10** demonstrates that at increasing distance from the detector, the higher energy ^{111}In (energy peaks at 171 and 245 keV) dominates detected count rates over the lower energy $^{99\text{m}}\text{Tc}$ (140.5 keV) implying that any alteration in the composition of the underlying lung tissue during the study period, such as may occur if the detector is repositioned, will alter the ratio of ^{111}In to $^{99\text{m}}\text{Tc}$ without the need for a change in pulmonary microvascular permeability.

| ^{99m} Tc window | ¹¹¹ In window | % spill over into |
|--------------------------|--------------------------|--------------------------|
| Count rate | Count rate | ¹¹¹ In window |
| 638 | 3 | 0.47 |
| 1153 | 12 | 1.04 |
| 447 | 3 | 0.67 |

Table 3.1 Count rate detected in the indium energy window of the Mediscint system from a point source of ^{99m}Tc placed in a waterbath.

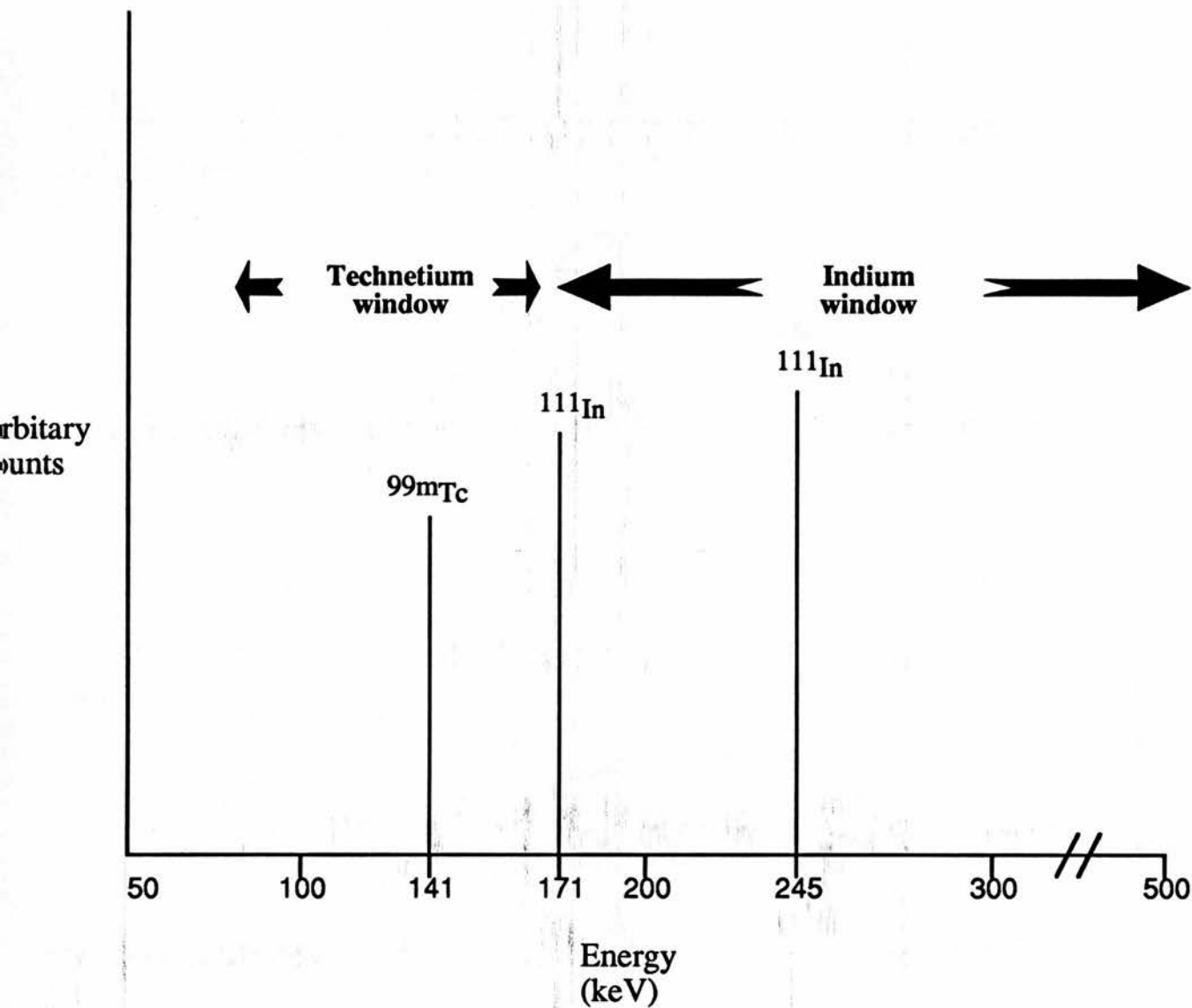


Figure 3.1 Calibration of the Mediscint Dual Isotope system. The lower energy window extends from 88 - 170 keV (to include the photopeak and compton scatter from ^{99m}Tc) and the upper from 170 - 500 keV (to include both photopeaks of ^{111}In and as much of the compton scatter radiation as possible).

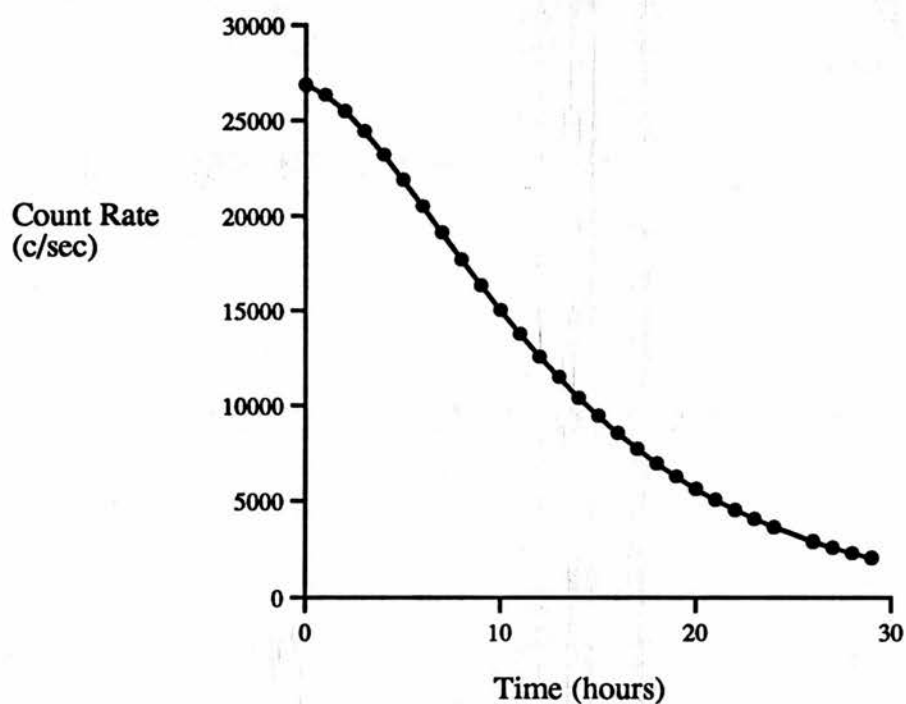


Figure 3.2 Graph of count rate observed by the Mediscint system from a point source of ^{99m}Tc at an initial energy sufficient to saturate the system. The point source was placed in a waterbath and count rates were recorded continuously over time.

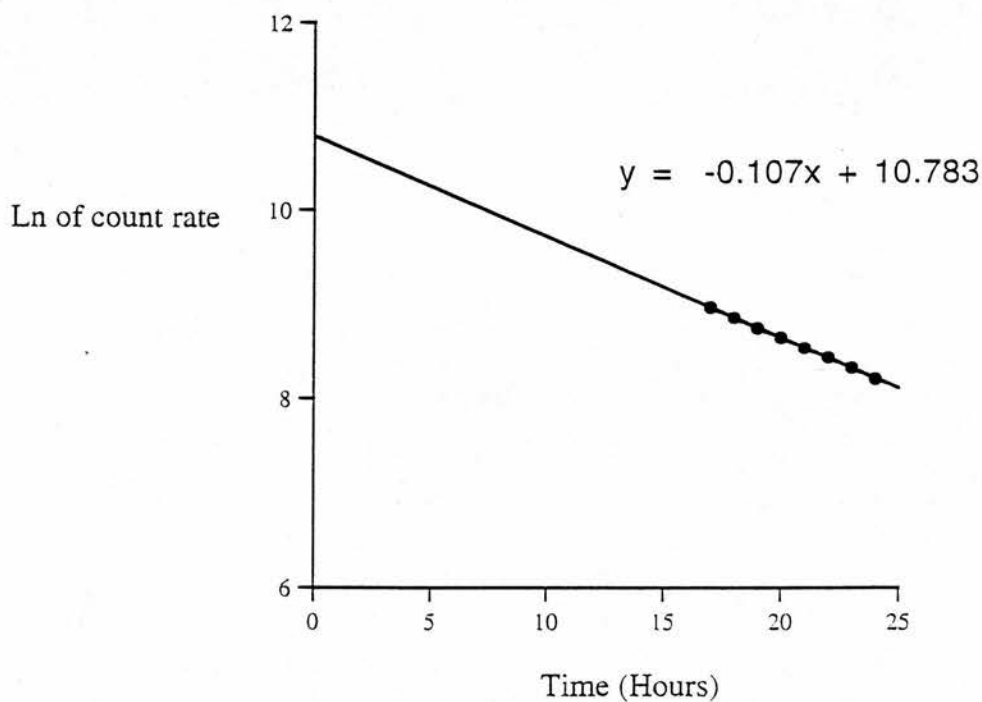


Figure 3.3 Graph of the natural log (ln) of the observed count rate plotted against time. Only the last 8 hours of the observed count rate are selected and the line of best fit is extrapolated to its intercept on the y axis. The expected count rates for each time period may then be determined from the formula shown.

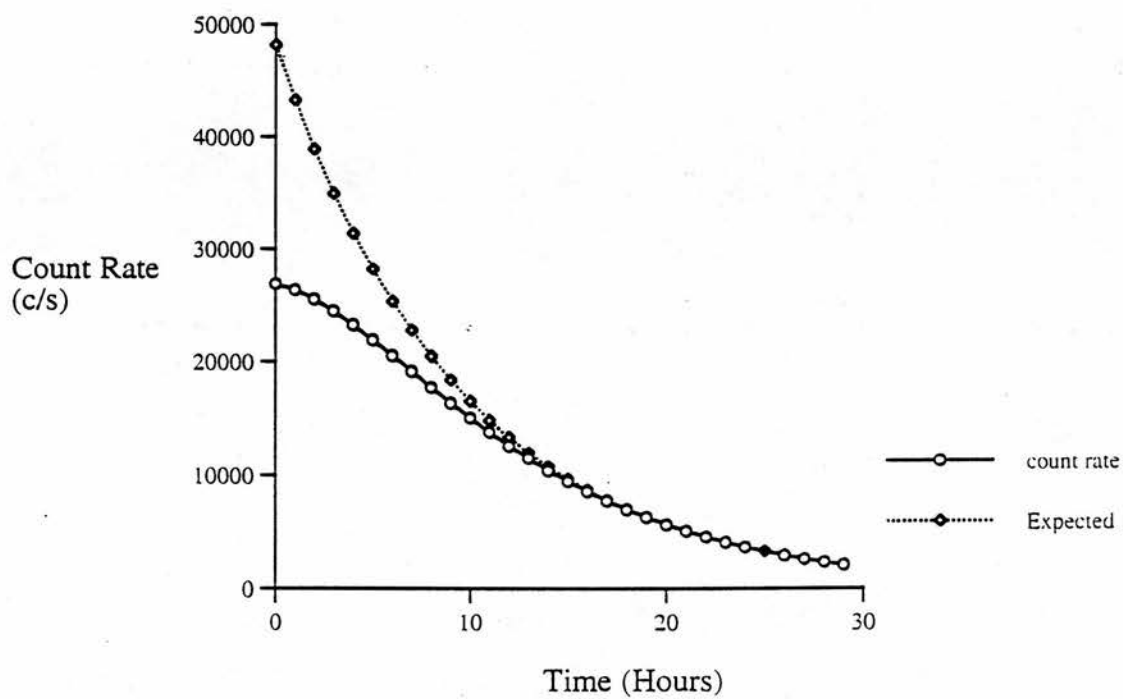


Figure 3.4 Graph of observed and expected count rates plotted against time. It can be seen that at higher count rates the observed counts are lower than expected reflecting dead-time losses.

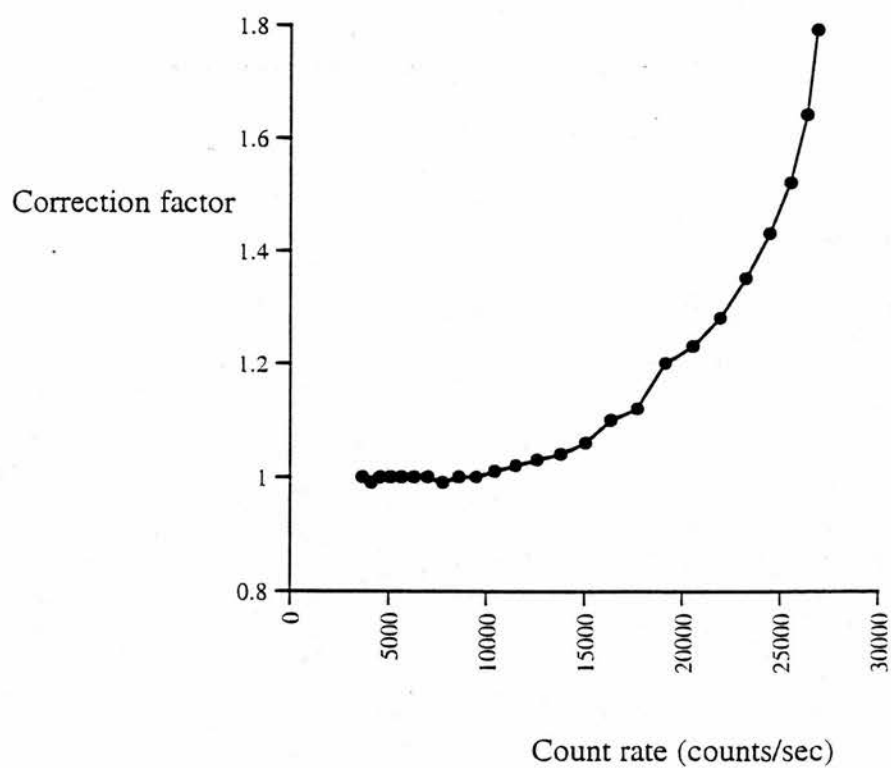


Figure 3.5 Graph of correction factor plotted against observed count rate. It can be seen that for count rates below 10 000 counts/sec no correction is required for dead-time loss.

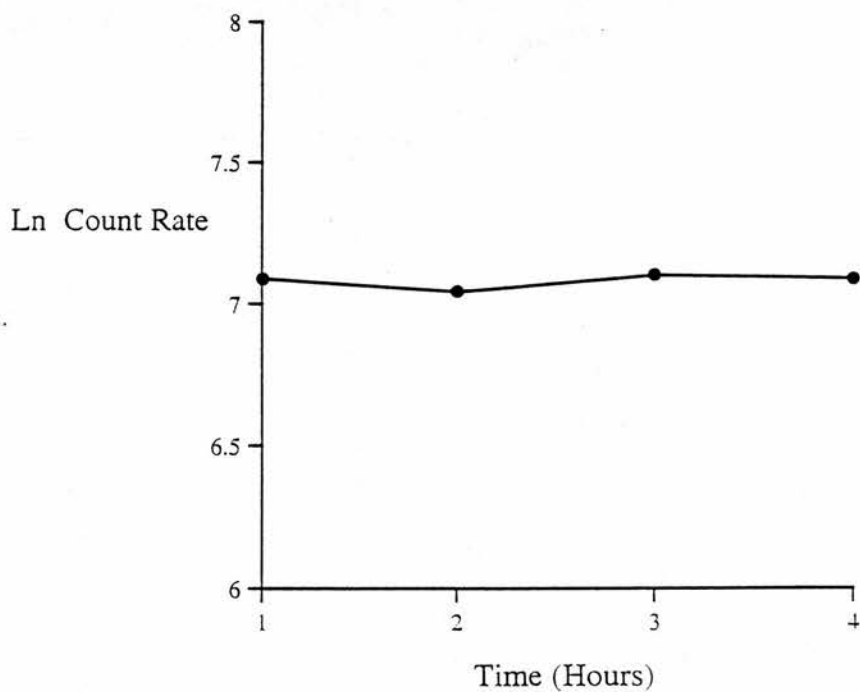


Figure 3.6 . Natural Log of corrected count rate from a point source of $^{99\text{m}}\text{Tc}$ plotted against time. No significant electrical drift occurs over the time period of the study.

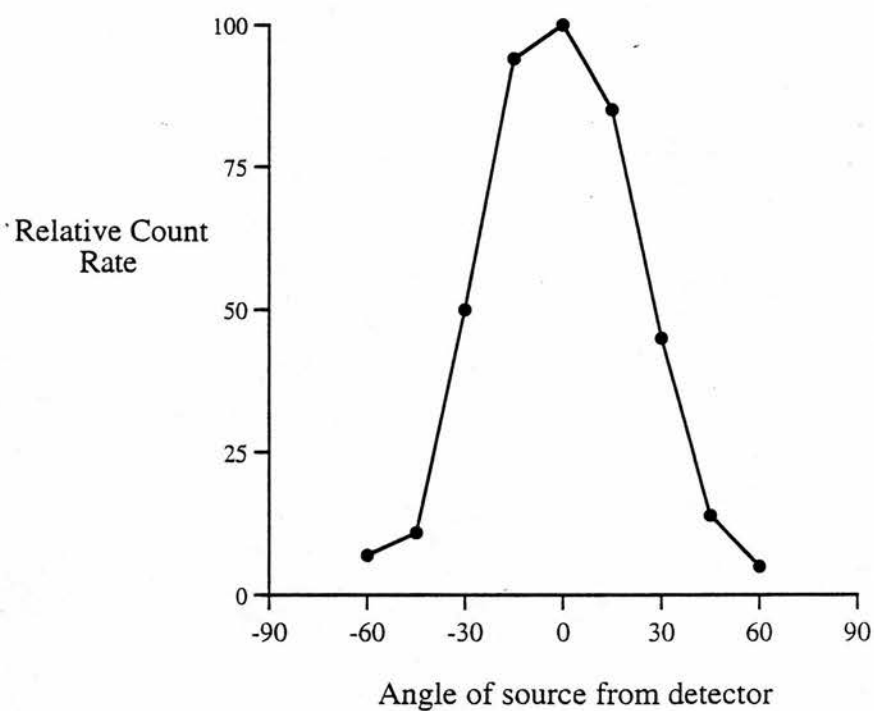


Figure 3.7 Relative Count Rates at differing angles from the detector from a point source of ^{111}In . It can be seen that the detected count rate rapidly falls as the point source is positioned at an angle of greater than 30° from the detector

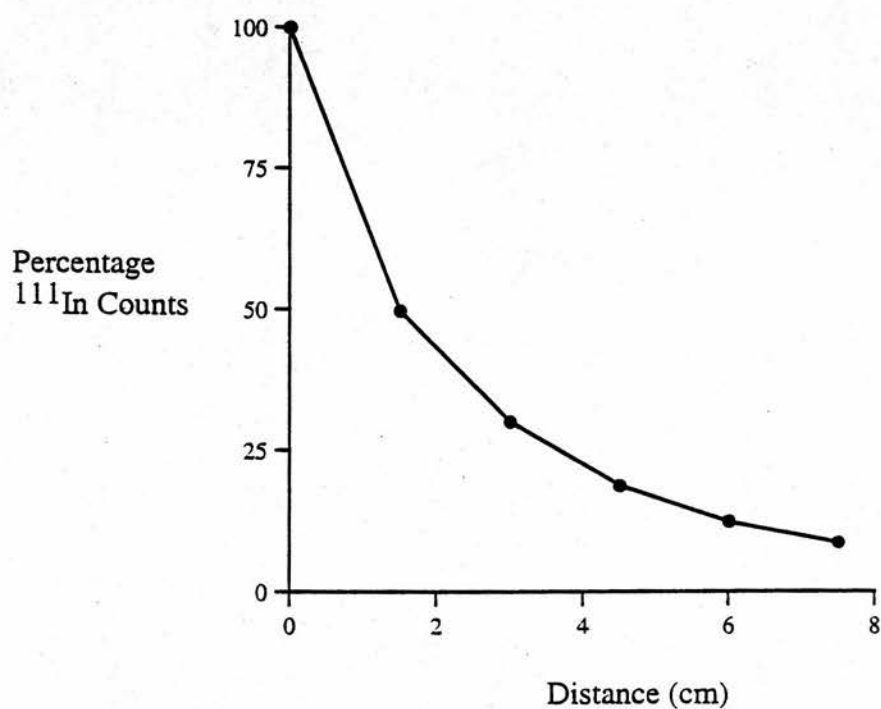


FIGURE 3.8 Percentage count rates detected from point source of indium which has been placed in a water bath at increasing distances from a miniature scintillation detector.

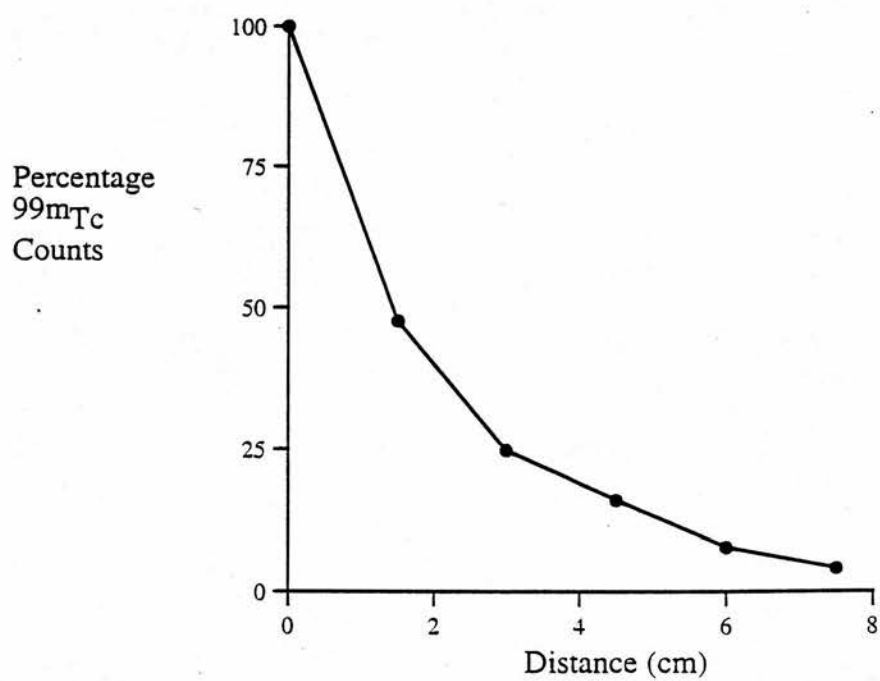


FIGURE 3.9 Percentage count rates detected from a point source of technetium which has been placed in a water bath at placed at increasing distances from a miniature scintillation detector.

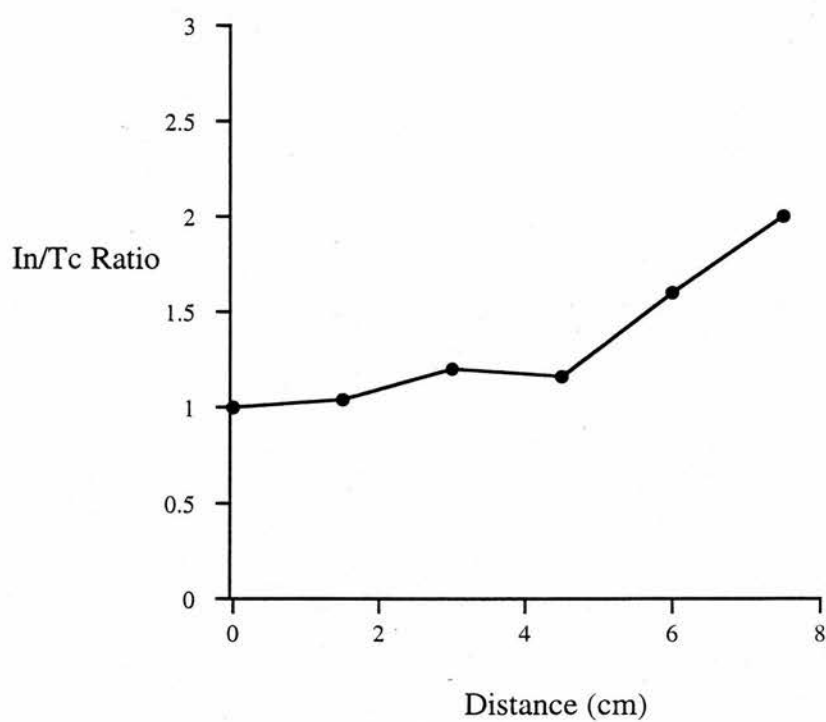


FIGURE 3.10 Ratio of In/Tc counts recorded from point sources of each isotope in a water bath. It can be seen that with increasing distance from the detector the higher energy indium dominates the detected count rate.

PATIENTS WITH ARDS

4.1 INTRODUCTION

As discussed in the introductory chapter, the measurement of pulmonary permeability is likely to be of benefit in both the diagnosis and management of patients with ARDS. In this study I wished to employ the portable Mediscint system in the setting of a busy Intensive Therapy Unit to record pulmonary microvascular permeability readings from patients with established acute respiratory distress syndrome. In addition, I wished to determine whether any relationship existed between the degree of pulmonary permeability recorded and other concomitantly displayed physiological parameters of gas exchange and circulating parameters of inflammatory cell and endothelial activation/damage which might be more easily measured in a greater number of patients and therefore applicable to multicentre studies.

4.2 PATIENT CHARACTERISTICS

A control group of eight healthy male volunteers was enrolled, median age 59 years (range 51-66). No patient in the control group had a history of recent or past respiratory illness and all were non-smokers. Chest radiograph and spirometry were within normal limits (**Table 4.1**). Twelve patients with ARDS were identified using the criteria of the American-European consensus statement (**Table 4.2**) and enrolled

in the study. Five patients were male, the median age was 71 years (range 49 - 83) and the median duration of ARDS was 6 days (range 1 - 15).

The median PAI for patients with established ARDS was $0.42 \times 10^{-3}/\text{min}$ (range -0.77 to $1.48 \times 10^{-3}/\text{min}$) compared to median value of $-0.5 \times 10^{-3}/\text{min}$ (range -1.73 to $0.27 \times 10^{-3}/\text{min}$) for the control group (**Figure 4.1**). There was no difference between the right and left lungs for both the control (**Figure 4.2**) and ARDS patient group (**Figure 4.3**) but the degree of correlation between both lungs was less good for the group of patients with ARDS with $r = 0.504$ compared to the correlation between right and left lungs of the normal volunteers with $r = 0.84$.

The median $\text{PaO}_2/\text{FiO}_2$ ratio was 21.9. (range 12.55 to 37.42). The median lung injury score was 3.25 (range 2.5 to 4.0).

Values for circulating markers were as follows. For the control group of healthy volunteers the circulating neutrophil elastase was 12.95ng/ml (range 8.4 to 19 ng/ml), thrombomodulin 24.7 ng/ml (range 3 to 47 ng/ml), vWf:Ag 1.03 IU (0.8 to 1.7 IU), sE-selectin 29.0 ng/ml (range 13.2 to 91 ng/ml), sP-selectin 150.5 ng/ml (range 78 to 263 ng/ml), sL-selectin 2.88 ng/ml (range 1.5 to 3.4 ng/ml).

For patients with ARDS the median and range for neutrophil elastase was 94.0 ng/ml (range 29 to 420 ng/ml), for thrombomodulin was 44.0 ng/ml (range 13.4 to 90.0 ng/ml), for vWf:Ag was 2.38 IU (range 0.75 to 4.80 IU), for sE-selectin was 75.0

(range 46 to 162 ng/ml), for sP-selectin was 92.0 ng/ml (range 40 to 118 ng/ml) and for sL-selectin 2.03 ng/ml (range 0.81 to 3.45). Compared to the control group, significantly altered levels of circulating Tm, vWf:Ag, neutrophil elastase, sE- and sP-selectin were observed (**Table 4.3**).

There was no relationship between the PAI and the gas exchange, lung injury score and any of the markers of neutrophil activation or endothelial activation/damage (**Table 4.4**).

4.3 SUMMARY OF RESULTS

This study showed greater pulmonary microvascular permeability in patients with established ARDS as compared to the control group. However considerable overlap occurs between the two groups and accordingly there was no statistical significant difference between patients with ARDS and the control group. There is no statistical difference between the PAI for each lung in both patient groups but the correlation between the right and left lungs is less good for patients with ARDS. No relationship was observed between the PAI and a physiological or circulating inflammatory marker.

4.4 DISCUSSION

Increased pulmonary microvascular permeability is believed to represent a fundamental pathophysiological event in the ARDS disease process and previous studies using a similar technique have demonstrated significant elevations in the protein accumulation index of patients with ARDS (Basran GS et al. 1985, Braude S et al. 1986, Rocker et al. 1988 (a)). Reasons for the limited elevation observed in this study may relate to the time point in the natural history of the disease at which the studies were performed or limitations in the recording system.

Previous studies were performed on patients following admission to the Intensive Care Unit and as soon as the diagnostic criteria for ARDS were fulfilled and thus it is likely that patients studied were in the exudative phase of the disease process. In this study the majority of patients were suffering from established disease with a median duration of 3 days (range 1, 15 days). Although it has been suggested that pulmonary microvascular permeability is elevated throughout the course of the ARDS disease process (Sinclair et al. 1994), histological examination of tissue obtained from patients with ARDS suggests that the condition evolves through an exudative phase of oedema and haemorrhage, a proliferative phase of organisation and repair and a fibrotic phase of end-stage fibrosis (Tomashefski et al. 1990, Hill et al. 1976). The pathology of established ARDS is characterised by the proliferation of fibroblasts and the deposition of new connective tissue matrix resulting in areas of damaged and scarred lung where protein leakage is not a dominant feature (Bachofen and Weibel. 1982). None of our patients underwent open lung biopsy but we might reasonably hypothesise that the majority of patients had made the transition from the

early exudative stage and would not necessarily be expected to display large increases in microvascular permeability. Both Braude and Rocker performed their studies on patients on admission to the Intensive Care Unit and as soon as the diagnostic criteria for ARDS were fulfilled and would thus have studied patients in the early and exudative stage of the disease. However, even at this time point both investigators recorded permeability indices in some ARDS patients within the normal range suggesting that there may be patients in whom ARDS may arise in the absence of any increase in pulmonary microvascular permeability, or that the phase of enhanced permeability was of short duration and had ceased before these measurements were taken.

In view of previous studies using similar equipment (Sinclair et al. 1994) and a PET scanning technique (Velaquez et al. 1991) demonstrating a relationship between the degree of lung leak and markers indicative of the severity of lung damage, it is disappointing that no such relationship was observed in this study. Pulmonary microvascular permeability recorded by a dual isotope system similar to that used in this thesis demonstrated a correlation between the protein accumulation index and both the lung injury score and the degree of neutrophilia on bronchoalveolar lavage in 14 patients with ARDS (Sinclair et al. 1994). In a dog model of inflammatory lung injury, regional pulmonary permeability assessed with a PET scanner could be correlated with the structural abnormality observed in the alveoli under both light and electron microscopy (Velaquez et al. 1991).

The use of miniature scintillation counters depends on the assumption that abnormalities of pulmonary permeability are homogeneously distributed throughout the lung and thus it will be possible to extrapolate from measurements made on a small volume of lung tissue to events occurring in the entire lung (Gorin et al. 1980). However the use of CT scanning has clearly demonstrated that in contrast to appearances on the chest radiograph, the distribution of the ARDS is far from homogenous. A study of 13 patients with ARDS, 11 patients in whom the chest radiograph demonstrated homogenous disease, CT examination revealed a patchy involvement with areas of relatively normal lung interspersed between patches of densely consolidated and collapsed lung (Maunder et al. 1986). This may be supported in these studies by finding that the degree of correlation between the right and left lung is less good for the patient group suffering from ARDS than the normal volunteers.

The use of a portable system with miniature scintillation probes instead of a gamma camera, whilst convenient, may give rise to sampling errors. In patients who had undergone re-expansion of lung during treatment for pleural effusion or pneumothorax, large sodium-iodide scintillation probes positioned over the area of presumed maximum re-expansion (as judged by the chest radiograph) compared to simultaneous data acquisition from a gamma camera, demonstrated that the probe system reported an erroneously low reading in just under one-third of patients. This was attributed to problems positioning the probe over the site of maximum increased permeability as detected by the gamma camera (Keegan et al. 1989).

Previous investigators have used the larger sodium-iodide detectors which although are both bulky and heavy, have the advantage that they may be focused to sample a greater depth of lung tissue (Gorin et al. 1981, Rocker et al 1988 (a) Rocker et al 1988 (b), Rocker et al 1989). The mediscint scintillation counter is essentially unfocused and as demonstrated in chapter three, is likely to sample only a limited volume of lung tissue which is unlikely to be representative of the ARDS disease process in the lung as a whole. Thus indicators of more generalised function such as gas exchange and lung injury score would be less likely to display a relationship with the PAI recorded by this technique. Similarly this may explain a lack of relationship with markers of endothelial damage.

These limitations may be obviated by use of regional imaging with a gamma camera, but this would result in the inconvenience of gamma camera recordings in the intensive care unit coupled with the requirement for larger doses of radioactivity. In considering future studies with these, or similar, miniature scintillation detectors, multiple recordings from the lung fields should be considered with investigators making recordings on the lateral and posterior chest walls. Given that the reticuloendothelial system is involved in clearing the labelled red cells it would be necessary to avoid counting over the liver and spleen and care must be taken with the left lung probe to avoid counting over the heart and major blood vessels. Centres with access to PET scanners may be better placed to answer the questions of this study.

A further factor to explain the lack of relationship with indices of lung injury may be attributed to the power of the study, given low patient numbers, to detect such a relationship. Accepting a power of $p < 0.1$ would demonstrate a relationship between the LIS and the PAI.

The factors discussed above may also explain the lack of relationship observed between the PAI and circulating markers of neutrophil activation or endothelial activation/damage, however, for future studies it should be considered whether other markers may be more appropriate. Given that pathological studies in ARDS often show greater epithelial than endothelial damage it may be helpful for future investigations to explore the relationship between the degree of pulmonary permeability and markers which specifically reflect epithelial damage. It is now possible to measure the serum concentration of surfactant protein A (SP-A), the major surfactant protein produced by type II pulmonary epithelial cells (Doyle et al. 1994) and recent work has begun to characterise markers of type I epithelial cell damage in animals (Dobbs et al. 1988, McElroy et al. 1995).

Circulating concentrations of SP-A have been examined in the sera from 15 patients with ARDS, 10 patients with acute cardiogenic pulmonary oedema, 6 ventilated patients with no cardiorespiratory disease and 15 healthy subjects. Significantly elevated levels were reported in the ARDS group and were found to correlate inversely with the static respiratory compliance and systemic oxygenation of the

patients. The investigators speculate that this finding may reflect abnormal alveolar-capillary permeability (Doyle et al 1995). Using an animal model of lung injury, Dobbs and colleagues have identified a specific 40- to 42- kDa protein (rTI₄₀) located on the apical surface of rat type I pulmonary epithelial cells (Dobbs et al. 1988) which may be detected in the BAL fluid following the administration of *Pseudomonas aeruginosa*. The group have demonstrated that elevated levels of rTI₄₀ reflect both the severity of injury to type I cells but also the degree of impairment to the alveolar-capillary barrier function (McElroy et al. 1995).

In conclusion, the PAI in patients with established ARDS in this study was marginally greater than that of a control group but considerable crossover occurred between groups and consequently no statistical significant difference was noted. This may be due to the selection of patients in the later stages of the disease process in which lung leak may not be a major component of on-going lung injury. No relationship was observed between the recorded pulmonary microvascular permeability and physiological or circulating markers. Although this may reflect the limited volume of lung sampled by the miniature scintillation detector and the power of the study with limited patient numbers it should be considered that other circulating markers which reflect injury to the pulmonary epithelial surface may prove more relevant as direct or surrogate markers of alveolar-capillary permeability.

| Volunteer | Age | Sex | Chest Radiograph | PAI Right lung ($\times 10^{-3}/\text{min}$) | PAI Left lung ($\times 10^{-3}/\text{min}$) | PAI Average ($\times 10^{-3}/\text{min}$) |
|-----------|-----|-----|------------------|---|--|--|
| 1 | 51 | M | normal | 0.06 | -0.50 | 0.28 |
| 2 | 66 | M | normal | -1.68 | -1.78 | -1.73 |
| 3 | 55 | M | normal | 0.60 | -0.05 | -0.28 |
| 4 | 52 | M | normal | 0.3 | 1.18 | 0.74 |
| 5 | 60 | M | normal | -0.44 | -0.42 | -0.43 |
| 6 | 65 | M | normal | 0.80 | 0.90 | 0.70 |
| 7 | 62 | M | normal | -0.11 | -0.43 | -0.27 |
| 8 | 58 | M | normal | -0.10 | -0.02 | -0.06 |

Table 4.1 Characteristics of healthy volunteers. All volunteers were non-smokers had a normal physical examination, normal spirometry and had no recent history of chest infection.

| Patient | Age (yrs) | Sex | Aetiology | ARDS duration (days) | Lung injury score | PaO ₂ /FiO ₂ ratio | PAI right lung (x10 ⁻³ /min) | PAI left lung (x10 ⁻³ /min) | PAI average (x10 ⁻³ /min) |
|---------|--------------|-----|------------------------|-------------------------|----------------------|---|---|--|--|
| 1 | 49 | M | Post-oesophagostomy | 5 | 3.5 | 12.3 | -0.59 | -0.8 | -0.70 |
| 2 | 69 | F | Oesophageal rupture | 2 | 2.5 | 15.6 | 0.35 | 0.86 | 0.61 |
| 3 | 72 | F | Trauma | 7 | 3.5 | 21.6 | 1.45 | 0.08 | 0.77 |
| 4 | 70 | F | Septicaemia | 3 | 3.5 | 22.5 | 0.90 | -0.01 | 0.45 |
| 5 | 58 | F | Septicaemia | 3 | 3.5 | 13.2 | 0.30 | -0.40 | -0.05 |
| 6 | 73 | F | Septicaemia | 2 | 3.0 | 20.3 | 1.48 | | 1.48 |
| 7 | 60 | M | Trauma | 7 | 2.5 | 23.6 | 0.9 | 0.09 | 0.50 |
| 8 | 74 | M | Trauma | 2 | 3.0 | 18.7 | -0.20 | -0.40 | -0.30 |
| 9 | 76 | F | Perforated bowel | 3 | 3.5 | 12.6 | -0.85 | -0.69 | -0.77 |
| 10 | 83 | M | Septicaemia | 13 | 4.0 | 11.3 | 0.50 | 0.30 | 0.40 |
| 11 | 63 | F | Septicaemia | 15 | 3.0 | 21.8 | -0.20 | 0.43 | 0.12 |
| 12 | 78 | M | Post-abdominal Surgery | 1 | 2.5 | 13 | 1.00 | | 1.00 |

Table 4.2 Characteristic of patient group enrolled with ARDS.

| | Controls | ARDS | p value |
|----------------|-----------------------|-----------------------|----------------|
| | Median (range) | Median (range) | |
| Elastase | 12.95 (8.4 to 19) | 94.0 (29 to 420) | 0.01 |
| Thrombomodulin | 24.7 (3 to 47) | 44.0 (13.4 to 90.0) | 0.03 |
| vWf:Ag | 1.03 (0.8 to 1.7) | 2.38 (0.75 to 4.8) | 0.03 |
| sL-selectin | 2.88 (1.5 to 3.4) | 2.03 (0.81 to 3.45) | ns |
| sE-selectin | 29 (13.2 to 91) | 75.0 (46 to 162) | 0.02 |
| sP-selectin | 150.5 (78 to 263) | 92.0 (40 to 118) | 0.01 |

Table 4.3 Circulating levels of plasma elastase (ng/ml), thrombomodulin (ng/ml), vWf:Ag (IU), sL, sE and sP-selectin (ng/ml) in healthy volunteers and patients with ARDS.

| | Correlation | Significance |
|------------------------------------|-------------|--------------|
| | r = | p = |
| PaO ₂ /FiO ₂ | 0.33 | 0.26 |
| D(A-a)O ₂ | -0.26 | 0.39 |
| Elastase | 0.10 | 0.75 |
| vWf:Ag | -0.27 | 0.38 |
| Thrombomodulin | 0.02 | 0.95 |
| sL-selectin | -0.43 | 0.15 |
| sE-selectin | 0.14 | 0.66 |
| sP-selectin | 0.3 | 0.34 |

Table 4.4 Relationship between the protein accumulation index (PAI) and the indices of gas exchange and circulating levels of plasma elastase (ng/ml), thrombomodulin (ng/ml), vWf:Ag (IU/ml), sL, sE and sP-selectin (ng/ml) patients with ARDS.

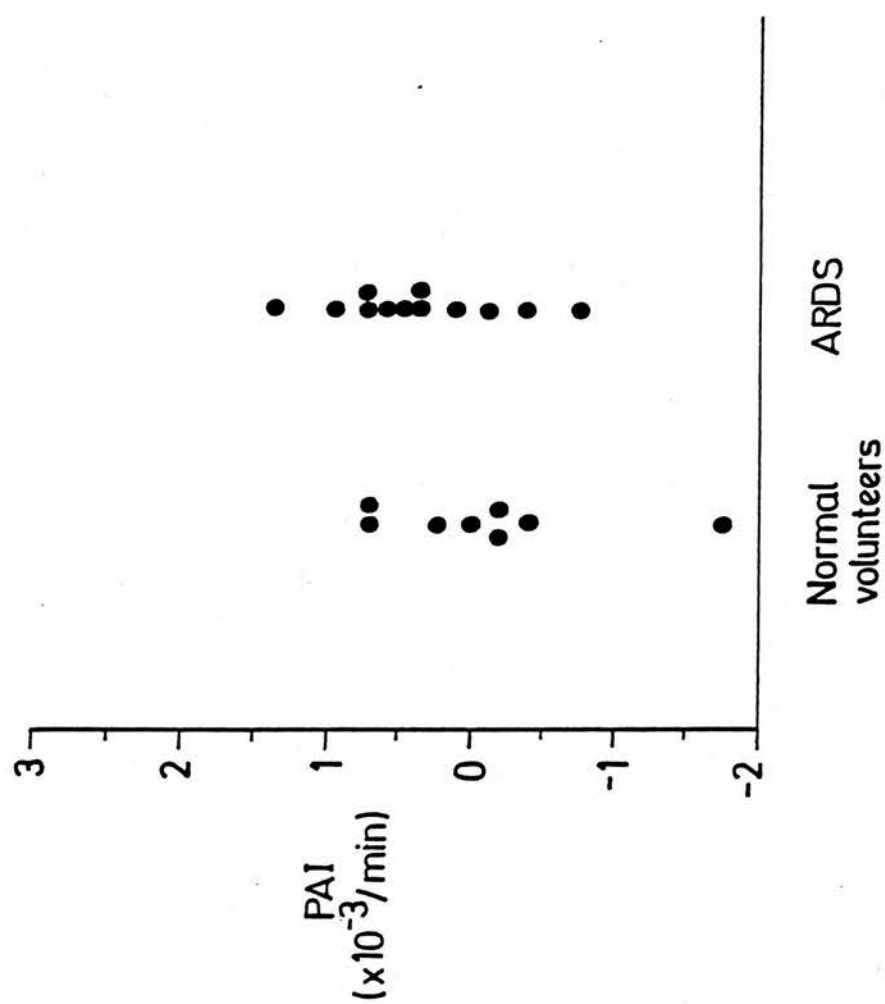


Figure 4.1 Protein accumulation indices (PAI) of healthy male volunteers, mean (SD) = $-0.11 (0.75) \times 10^{-3}/\text{min}$ and patients with ARDS, mean (SD) = $0.29 (0.67) \times 10^{-3}/\text{min}$. There is no statistical difference between the two groups.

PATIENTS AT-RISK OF ARDS

5.1 INTRODUCTION

As discussed in chapter one, the earliest pathophysiological event in the genesis of ALI/ARDS is extensive microvascular injury believed to be consequent upon an exuberant local inflammatory response in which adherent activated inflammatory cells (principally neutrophils) degranulate into a protected microenvironment, facilitating unopposed degradation of the delicate pulmonary endothelium. The leak of a protein rich fluid from the capillaries into the pulmonary interstitium and alveolar airspaces may be charted in a non-invasive manner utilising radiolabelled proteins and externally situated scintillation detectors. In chapter 4, studies utilising the portable Mediscint system in patients with established ARDS were described. However, for various reasons including the limited volume of lung sampled by the miniature scintillation detectors, it was concluded that the technique is unlikely to be suitable in patients with established ARDS in whom the disease process may be heterogeneous. In this chapter the use of the Mediscint system was investigated in patients earlier in the disease process, in whom the disease process may more homogenous.

The aim of this study was therefore to assess the use of the Mediscint system in patients at-risk of ALI/ARDS and again to determine if any relationship existed between such measurement and physiological or circulating markers of alveolar-capillary injury. I hypothesised that since the earliest events in the pathogenesis of

acute lung injury syndromes are predominantly neutrophil mediated and occur in the intravascular space, the most likely candidates for suitable markers reflecting an increase in pulmonary microvascular permeability would result from neutrophil activation and/or endothelial activation/injury. Markers particularly chosen for analysis were neutrophil elastase, sL-, sE- and sP-selectin, thrombomodulin and von-Willebrand factor antigen.

5.2 PATIENT CHARACTERISTICS

The control group have been documented in chapter 4. Twenty patients undergoing oesophagogastrrectomy for malignant disease were enrolled, 15 male. All patients had a normal pre-operative chest radiograph. The characteristics of the post-oesophagogastrrectomy patients are summarised in **Table 5.1**. The median age as 65.5 years (range 52, 79). Seventeen patients underwent thoracic resection via a left thoracotomy and three patients right thoracotomy and abdominal resection. The pulmonary microvascular permeability scans were carried out within six hours of the procedure. Prior to commencing the study the patients underwent a post-operative chest radiograph. The control group of healthy volunteers have been detailed in chapter 4.2.

The median post-operative $\text{PaO}_2/\text{FiO}_2$ ratio performed at the termination of the scan was 43.7 (range 23 to 75.8). The chest radiograph demonstrated minor atelectasis in

4 patients and small pleural effusion in 2 patients but no patient displayed bilateral diffuse shadowing consistent with acute lung injury.

The median PAI for the healthy male volunteers was $-0.5 \times 10^{-3}/\text{min}$ (range -1.73 to $0.27 \times 10^{-3}/\text{min}$). The median PAI for patients post-oesophagogastratomy was $-0.005 \times 10^{-3}/\text{min}$ (range -1.53 to $2.28 \times 10^{-3}/\text{min}$). There was no significant difference between these two groups (**Figure 5.1**). There was no significant difference in the PAI recorded from the lung ventilated during oesophagogastratomy and the lung which was deflated (**Figure 5.2**).

The circulating markers post-oesophagogastratomy were as follows. The median values of the circulating neutrophil elastase was 63.8 ng/ml (range 37 to 123 ng/ml), of thrombomodulin was 37.0 ng/ml (range 9.6 to 52 ng/ml), vWf:Ag 3.4 IU (range 1.3 to 6.2 IU), of sE-selectin was 74.5 ng/ml (41 - 105 ng/ml), sP-selectin 229 ng/ml (range 129 to 343 ng/ml) and sL-selectin 1.96 ng/ml (range 0.8 to 4.6 ng/ml). A significant difference was seen between the control group and the post-oesophagogastratomy group for the circulating plasma elastase, Tm, vWf:Ag, sE- and sP- selectin but there was no difference between the levels of sL-selectin (**Table 5.2**).

A significant relationship was observed between the PAI and the $\text{PaO}_2/\text{FiO}_2$ ratio ($p = 0.04$, $r = -0.46$)(**Figure 5.3**) and the circulating plasma elastase ($p = 0.04$, $r = 0.49$) (**Figure 5.4**). However there was no relationship between the PAI and the circulating

elastase in the normal volunteers. There was no relationship between the PAI and any of the other circulating markers assayed in the normal volunteers or patient group.(Table 5.3).

Patient numbers 1 and 9 developed persisting post-operative lung injury. Patient number 1 developed bilateral diffuse radiographic infiltrates on subsequent PA chest radiographs and a $\text{PaO}_2/\text{FiO}_2$ ratio of 16.9, 26.75 and 21.25 on the subsequent three post-operative days, fulfilling the American-European Consensus diagnostic criteria for both ALI and ARDS, before beginning to improve. He did not require ventilation. Patient number 9 could not be weaned from the ventilator and developed bilateral radiographic shadowing with $\text{PaO}_2/\text{FiO}_2$ ratios of 17.14, 12 and 22.8 again fulfilling the American-European Consensus diagnostic criteria for ARDS. Pulmonary capillary wedge pressure recorded on the fourth post-operative day was 22 mmHg. Patient number 4 suffered a collapse of the left lung 72 hours post-operatively and required suction with rigid bronchoscopy. Patient number 15 was thought to undergo aspiration on the second post-operative day. The remaining patients made uneventful recovery.

5.3 SUMMARY OF RESULTS

There is a trend towards elevation of the PAI in patients who have undergone oesophagogastrrectomy compared to the control group but this does not attain statistical significance. Several of the studies display a negative value of PAI.

Significant differences are observed between the control group and patients post-oesophagogastrrectomy for circulating plasma elastase, Tm, vWf:Ag, sE- and sP-selectin. A statistically significant relationship is demonstrated between the PAI and the $\text{PaO}_2/\text{FiO}_2$ ratio and the circulating plasma elastase however the relationship with elastase does not hold true when one considers the normal volunteer group.

5.4 DISCUSSION

Oesophagogastrrectomy is a recognised palliative procedure for malignant disease of the oesophagus and cardia of the stomach. The procedure is safe with an acceptable morbidity and mortality (Sabanathan et al 1996). The majority of early deaths relate to the post-operative development of respiratory failure the causes of which are multifactorial and include infection, aspiration, a systemic inflammatory response and in some cases ALI/ARDS. Oesophagogastrrectomy has previously been shown to cause an elevation in pulmonary microvascular permeability (Rocker et al. 1988 (b)) but the relationship between this and a range of physiological and inflammatory parameters has not been compared.

In this study only 5 patients developed a PAI which was greater than 1 SD above that of a control group and only 2 patients who developed an elevation in PAI greater than 2 SD. The majority of patients maintained a PAI comparable to that of the healthy volunteers and consequently there was no statistical difference between the

two groups. Only two patients progressed to develop ARDS. Patient number 1 although having a clear chest radiograph at the time of recording a PAI of 2.28×10^{-3} /min (ventilated lung 2.22 and deflated lung 2.34) on the following 3 post-operative days developed bilateral infiltrates on anteroposterior chest radiographs and fulfilled the American-European consensus criteria for ARDS. The patient did not require ventilation and a pulmonary artery wedge pressure was not recorded, although there were no other clinical signs of left atrial hypertension. Patient number one also recorded the lowest level of sL-selectin at 0.8 ng/ml and a high circulating elastase at 73.5 ng/ml. Patient number nine, with a PAI of 0.1×10^{-3} /min (ventilated lung 0.7, deflated lung -0.5), which fell within the normal range displayed by the control group, was unable to be weaned from the ventilator, rapidly developing bilateral infiltrates on anteroposterior chest radiographs and impaired systemic oxygenation consistent with a diagnosis of ARDS. The pulmonary artery wedge pressure was not recorded until the fourth post-operative day and was elevated at 22 mmHg, however for reasons we have discussed in chapter 1, this does not necessarily imply the clinical picture is secondary to cardiogenic oedema. Patient number nine also displayed the highest levels of circulating elastase at 101 ng/ml and a low sL-selectin of 1.18 ng/ml. In contrast, patient number 20, with a PAI of 1.9×10^{-3} /min made an uncomplicated recovery. The circulating elastase in patient number 20 was elevated at 84 ng/ml as was the sL-selectin was marginally elevated at 2.28 ng/ml. Thus the protein accumulation index, per se, as measured by this system is unable to predict progression to ARDS. However there does appear to be a relationship between the post-operative $\text{PaO}_2/\text{FiO}_2$ ratio and the PAI which suggests that the degree of

endothelial permeability is a component of the early post-operative changes observed in gas exchange.

Although there has been some speculation that an index of pulmonary endothelial permeability may be helpful in predicting progression to ARDS (Braude S et al 1992), this may be considered overly optimistic. The pulmonary epithelial barrier is recognised to represent the rate limiting step preventing alveolar oedema (Schneeberger EE 1979) and, primarily via a sodium transport pump, is responsible for reabsorption of any flooding which does occur (Mason et al. 1982, Matthay and Wiener-Kronish. 1990). Furthermore animal models of acute lung injury have demonstrated recovery of both the endothelial and epithelial barriers within several hours of an antecedent precipitant to a level sufficient to facilitate reabsorption of alveolar fluid (Wiener-Kronish et al 1991, Wiener-Kronish et al 1993, Pittet et al. 1995). Thus transient increases in permeability, as seen in patient 20, may represent a *forme fruste* rather than a forerunner of inflammatory injury.

The limited role of the endothelial barrier is also supported in clinical studies where endothelial markers have so far failed to identify those patients who may subsequently progress to ARDS. Although one study of patients with non-pulmonary sepsis found that an elevated plasma level of vWf:Ag had the potential to identify patients who would subsequently progress to ARDS (Rubin et al. 1990), this has not been borne out by further studies on more diverse patient groups (Moss et al. 1995, Sabharwal et al. 1995), although it is possible that the pathogenesis of lung

injury may differ according to the antecedent precipitant (Moss et al 1996). In addition, we have also found circulating levels of E- and P-selectin and Tm to be unhelpful (Donnelly et al. 1994, Reid PT et al. 1995 (a)).

Attempts to identify a circulating marker to represent a surrogate for pulmonary permeability continue to prove elusive. In this study a statistically significant correlation was observed between the pulmonary microvascular permeability recorded by the Mediscint system and the circulating neutrophil elastase in the post-oesophagogastrectomy patient group. However, this relationship does not hold within the group of normal volunteers. Further larger studies will be required to clarify whether this relationship may prove to be valid. One previous study including a more diverse patient group found a relationship between the PAI as measured by a similar dual isotope technique and both the circulating and free elastase in the BAL (Rocker et al. 1989) and in a study of fourteen patients with ARDS a relationship was observed between the PAI and the BAL neutrophil count (Sinclair et al. 1994). Furthermore, our group has recently demonstrated that the initial plasma elastase, taken in the resuscitation room of patients suffering multiple trauma may assist in the identification of those patients who subsequently progress to ARDS (Donnelly et al. 1995). Nevertheless, the plasma elastase level in the post-oesophagogastrectomy group only accounts for 25% of the variability and other factors undoubtedly influence endothelial permeability including other neutrophil serine proteases such as cathepsin G (Peterson. 1988) and free radicals such as hydrogen peroxide and nitric oxide (Zhao et al. 1998, Okayama et al. 1997).

The post-operative levels of the endothelial markers were significantly elevated consistent with endothelial activation and/or damage but again no relationship was demonstrated between these markers and the pulmonary microvascular permeability recorded by our system. Before discounting any possible relationship it is perhaps worth speculating that the choice of patient group was inappropriate to detect such a relationship. Surgical resection of the oesophageal malignancy and fashioning of the oesophagogastric anastomosis may result in elevation of endothelial markers per se, in addition to those which may be released from the pulmonary circulation, which could mask any relationship which may exist between these markers and the PAI. In order to clarify this endothelial markers which are specific to either the pulmonary or systemic circulation would be required. Of the currently available endothelial markers, both vWf:Ag and sP-selectin are not only expressed throughout the endothelial surfaces of the body but are also released from platelets (Sporn et al. 1985, Hsu-Lin et al. 1984, McEver et al. 1989). E-selectin and Tm appear to be specific for the endothelium, but are widely distributed throughout the endothelium of the body (Newman et al. 1993, Maruyama et al. 1985).

Finally one must speculate that this patient group may also act to confound the measurement of PAI itself. In these studies many patients displayed a negative value for the PAI post-oesophagogastricectomy. Negative values have been recorded by previous investigators using different isotopes and have been observed in studies by other investigators (Basran et al 1985, Rocker et al 1987, Rocker et al 1988a).

Negative values can potentially arise from inefficient binding of the ^{99m}Tc to the red blood cells. This is an unlikely reason in these studies as a minimum of fifteen minutes was allowed from the administration of the stannous agent to the administration of the ^{99m}Tc . The most likely explanation in these studies is that the fashioning of the oesophagogastric anastomosis results in an inflammatory response with an associated microvascular leak which is detected by the heart probe. This explanation is supported by examining the slope of the heart indium trace. Examples of post-oesophagogastric anastomosis patients in which a positive slope is observed are shown (**figures 5.5 - 5.8**). **Figures 5.9 and 5.10** provide examples of the curves obtained from normal volunteers and **figures 5.11 - 5.14** examples of curves obtained from patients with ARDS. Furthermore the median slope index for heart indium in normal volunteers is $-0.48 \times 10^{-3}/\text{min}$ (range -1.8 to 1.0), for patients with ARDS is $-3.3 \times 10^{-3}/\text{min}$ (range -2.93 to -1.02) but for patients post-oesophagogastric anastomosis is $-0.17 \times 10^{-3}/\text{min}$ (range -4.4 to 3.92). Although this patient group has been studied before without this apparent difficulty this may reflect the fact that a focused collimator was used (Rocker et al 1998 b) in contrast to the unfocused collimator in this equipment. Furthermore, we had increased the window settings to maximise count rates from ^{111}In and as much of the Compton scattered radiation as possible (Chapter 3.1.1) which may have contributed to this phenomenon. In order to correct for this in future studies it should be possible to modify the protocol. In studies from the Netherlands determination of the circulating radionuclide activity was made by serial venous blood sampling throughout the study period (Raijmakers et al 1993, Raijmakers et al 1995). Whilst this has the disadvantage of requiring repeated

transport of radioactive blood from the patient to a suitable radionuclide calibrator, it eliminates any potential error arising from the collection of additional count rates by the heart probe. This is of further importance when one considers patients undergoing cardiopulmonary bypass provide an attractive model for studies (MacNaughton et al. 1992). It is very likely that the median sternotomy and any associated procedures such as harvesting of the internal mammary artery would give rise to similar problems.

In conclusion patients post-oesophagogastrrectomy develop a moderate inflammatory response and a limited number display a modest elevation in pulmonary endothelial permeability but this does not in itself appear to predict progression to acute lung injury although it does demonstrate correlation with the post-operative gas-exchange suggesting that early post-operative abnormalities in gas exchange may be due to enhanced endothelial permeability. A convincing surrogate marker for pulmonary endothelial permeability remains elusive.

| Patient | Age (yrs) | Sex | Diagnosis | PaO ₂ /FiO ₂ | PAI Deflated lung | PAI Ventilated lung | PAI (mean) | CXR appearance |
|---------|--------------|-----|----------------|------------------------------------|----------------------|------------------------|---------------|-------------------------------|
| 1 | 74 | M | Adenocarcinoma | 23.00 | 2.22 | 2.34 | 2.28 | minor right basal atelectasis |
| 2 | 66 | M | Adenocarcinoma | 75.13 | -1.686 | -0.3 | -0.993 | clear lung fields |
| 3 | 55 | M | Adenocarcinoma | 33.13 | 0.38 | 1.428 | 0.906 | minor bilateral atelectasis |
| 4 | 77 | M | Adenocarcinoma | 42.89 | 0.24 | -0.46 | -0.11 | minor left basal atelectasis |
| 5 | 72 | M | Adenocarcinoma | 52.55 | -1.6 | -1.09 | -1.34 | clear lung fields |
| 6 | 65 | M | Adenocarcinoma | 51.33 | -0.59 | -0.6 | -0.595 | small left effusion |
| 7 | 58 | M | Adenocarcinoma | 47.21 | -1.86 | -1.2 | -1.53 | clear lung fields |
| 8 | 52 | M | Adenocarcinoma | 75.80 | | -0.754 | -0.754 | clear lung fields |
| 9 | 69 | F | Squamous cell | 35.76 | 0.7 | -0.5 | 0.1 | clear lung fields |
| 10 | 65 | M | Adenocarcinoma | 44.65 | -0.25 | -1.46 | 0.855 | clear lung fields |
| 11 | 61 | M | Adenocarcinoma | 45.26 | 1.11 | 0.768 | 0.939 | clear lung fields |
| 12 | 70 | M | Squamous cell | 38.76 | -0.03 | -1.3 | -0.665 | clear lung fields |
| 13 | 68 | F | Adenocarcinoma | 59.03 | -0.02 | 0.0438 | 0.01 | minor left basal atelectasis |
| 14 | 70 | F | Adenocarcinoma | 74.52 | -0.6 | 1.1 | 0.25 | clear lung fields |
| 15 | 65 | M | Melanoma | 38.55 | -0.17 | 0.9 | 0.365 | minor left basal atelectasis |
| 16 | 68 | F | Adenocarcinoma | 42.00 | 0.17 | -1.41 | -0.62 | clear lung fields |
| 17 | 59 | M | Adenocarcinoma | 38.00 | -0.91 | -0.44 | -0.675 | small left pleural effusion |
| 18 | 53 | M | Adenocarcinoma | 38.00 | -0.6 | | -0.6 | clear lung fields |
| 19 | 79 | F | Adenocarcinoma | 61.12 | 0.15 | -0.06 | 0.045 | clear lung fields |
| 20 | 65 | M | Adenocarcinoma | 35.60 | 1.9 | | 1.9 | clear lung fields |

Table 5.1 Characteristics of patients enrolled post-oesophagogastrctomy. PAI (protein accumulation index with unit 10⁻³/min)

| | Normal | Post- oesophagogastrectomy | |
|----------------|-------------------|-------------------------------|---------|
| | median (range) | median (range) | p value |
| Elastase | 12.95 (8.4 to 19) | 63.8 (37 to 123) | <0.01 |
| Thrombomodulin | 24.7 (3 to 47) | 37 (9.6 to 52) | 0.04 |
| vWf:Ag | 1.03 (0.8 to 1.7) | 3.4 (1.3 to 6.2) | 0.01 |
| sL-selectin | 2.88 (1.5 to 3.4) | 1.96 (0.8 to 4.6) | ns |
| sE-selectin | 29 (13.2 to 91) | 74.5 (41 - 105) | 0.03 |
| sP-selectin | 150.5 (78 to 263) | 229 (129 to 343) | 0.02 |

Table 5.2 Circulating levels of plasma elastase (ng/ml), thrombomodulin (ng/ml), vWf:Ag (IU), sL, sE and sP-selectin (ng/ml) in healthy volunteers and post-oesophagogastrectomy patients.

| | Correlation | Significance |
|----------------|--------------------|---------------------|
| | r = | p = |
| vWf:Ag | 0.16 | 0.48 |
| Thrombomodulin | -0.12 | 0.61 |
| sL-selectin | -0.13 | 0.57 |
| sE-selectin | -0.22 | 0.39 |
| sP-selectin | 0.37 | 0.11 |

Table 5.3 Relationship between the protein accumulation index measured by the Mediscint system and circulating levels of thrombomodulin (ng/ml), vWf:Ag (IU), sL, sE and sP-selectin (ng/ml) patients post-oesophagogastrectomy.

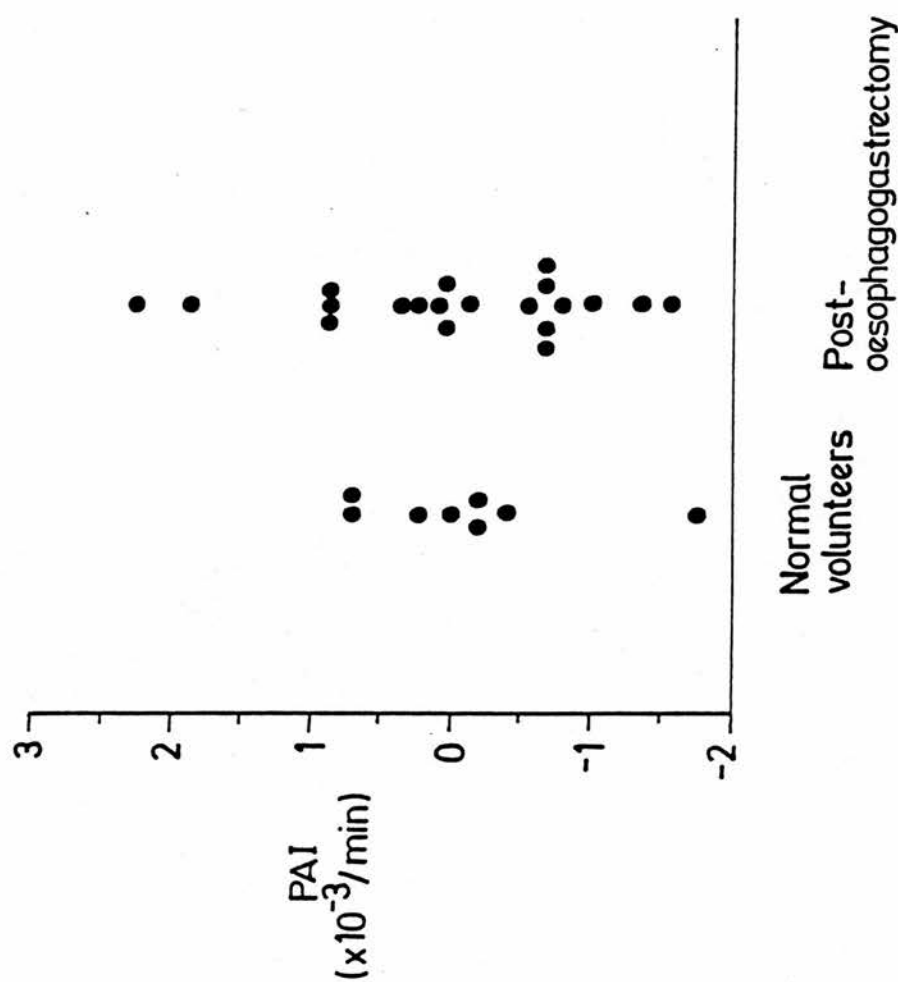


Figure 5.1 Protein accumulation indices (PAI) of healthy male volunteers, mean (SD) = -0.11 (0.75) $\times 10^{-3}/\text{min}$ and patients post-oesophagogastrectomy, mean (SD) = -0.01 (1.0) $\times 10^{-3}/\text{min}$. Considerable overlap occurs and accordingly there is no statistical difference between the two groups.

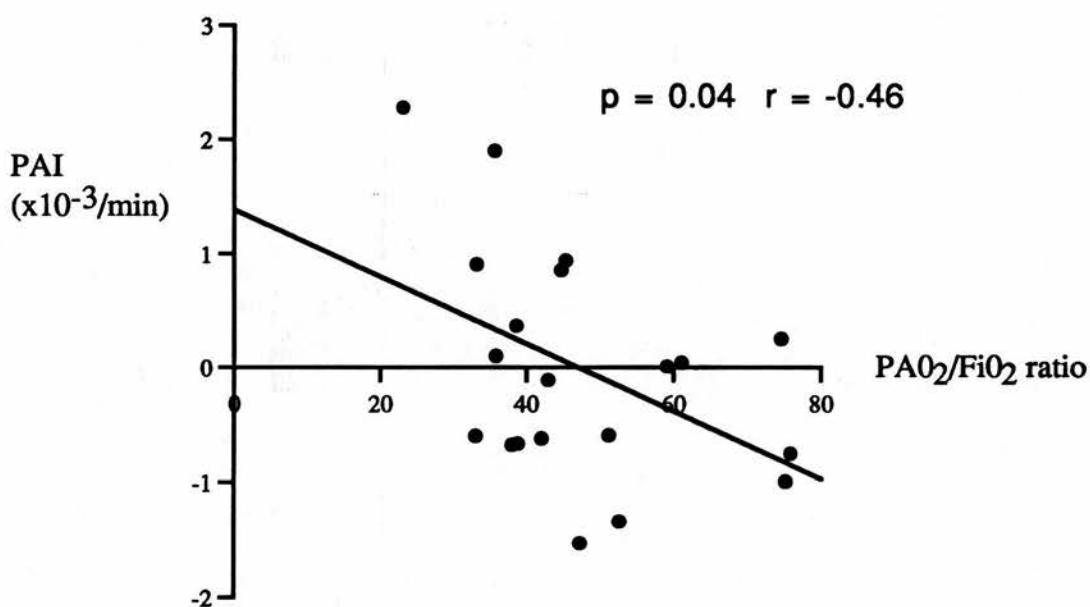


Figure 5.3 Relationship between the PAO_2/FiO_2 ratio and the protein accumulation index (PAI) measured in patients post-oesphagogastrrectomy.

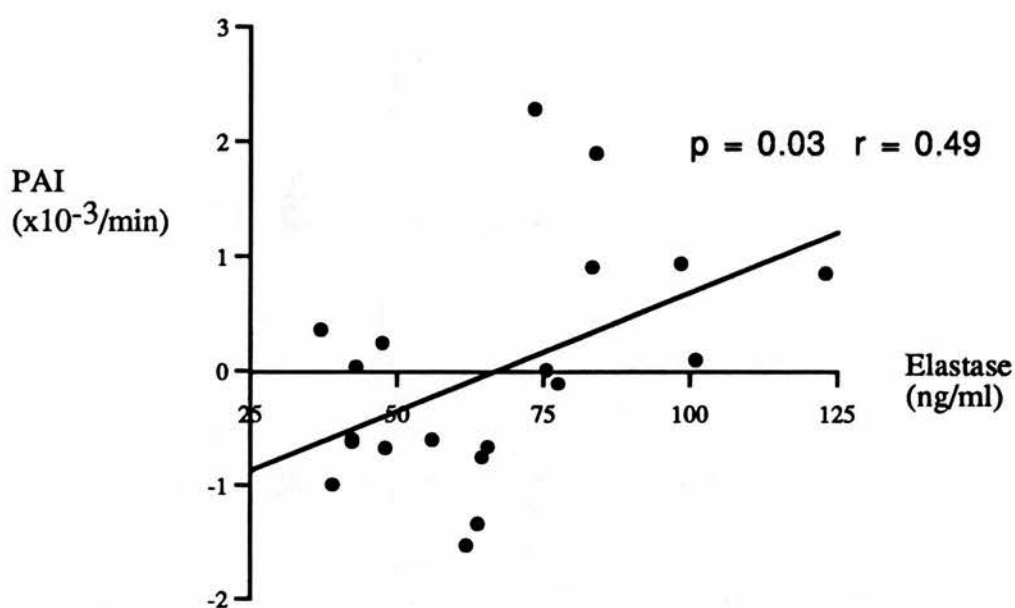


Figure 5.4 . Relationship between the the protein accumulation index (PAI) and the circulating plasma elastase (ng/ml) post-oesophagogastrrectomy.

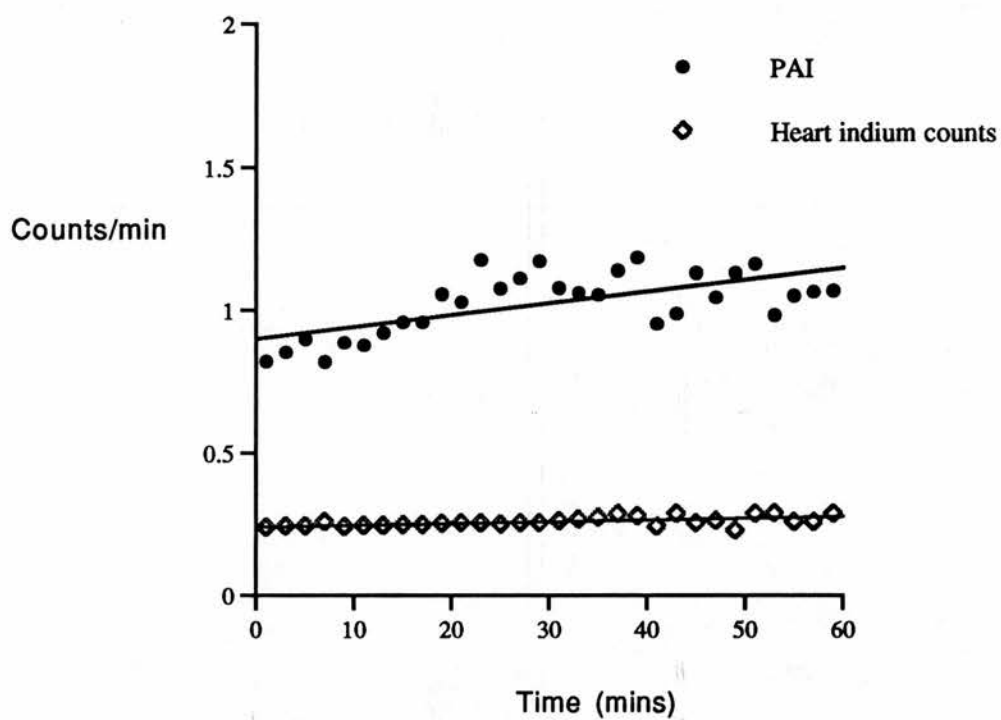


Figure 5.5 Graph of recordings from post-oesophagogastrrectomy patient number 1. The PAI = $2.28 \times 10^{-3}/\text{min}$ and the slope of the heart indium = $1.02 \times 10^{-3}/\text{min}$.

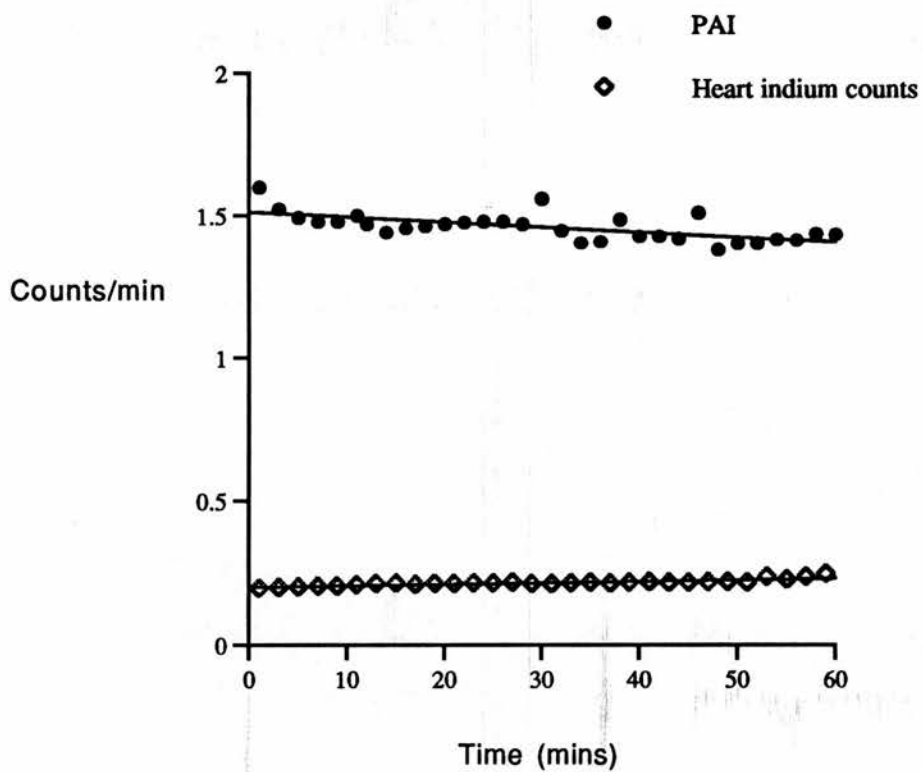


Figure 5.6 Graph of recordings from post-oesophagogastrrectomy patient number 5. The PAI = $-1.34 \times 10^{-3}/\text{min}$ and the slope of the heart indium curve is $1.7 \times 10^{-3}/\text{min}$.

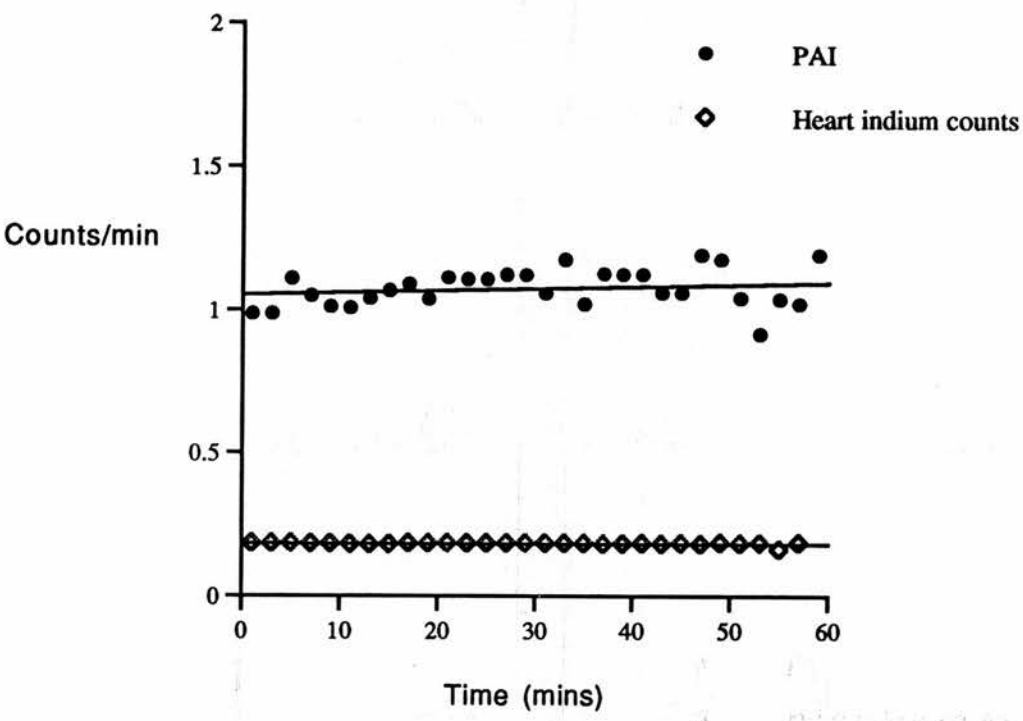


Figure 5.7 Graph of recordings from post-oesophagogastrrectomy patient number 11. The PAI = $0.94 \times 10^{-3}/\text{min}$ and the slope of the heart indium trace is $0.53 \times 10^{-3}/\text{min}$.

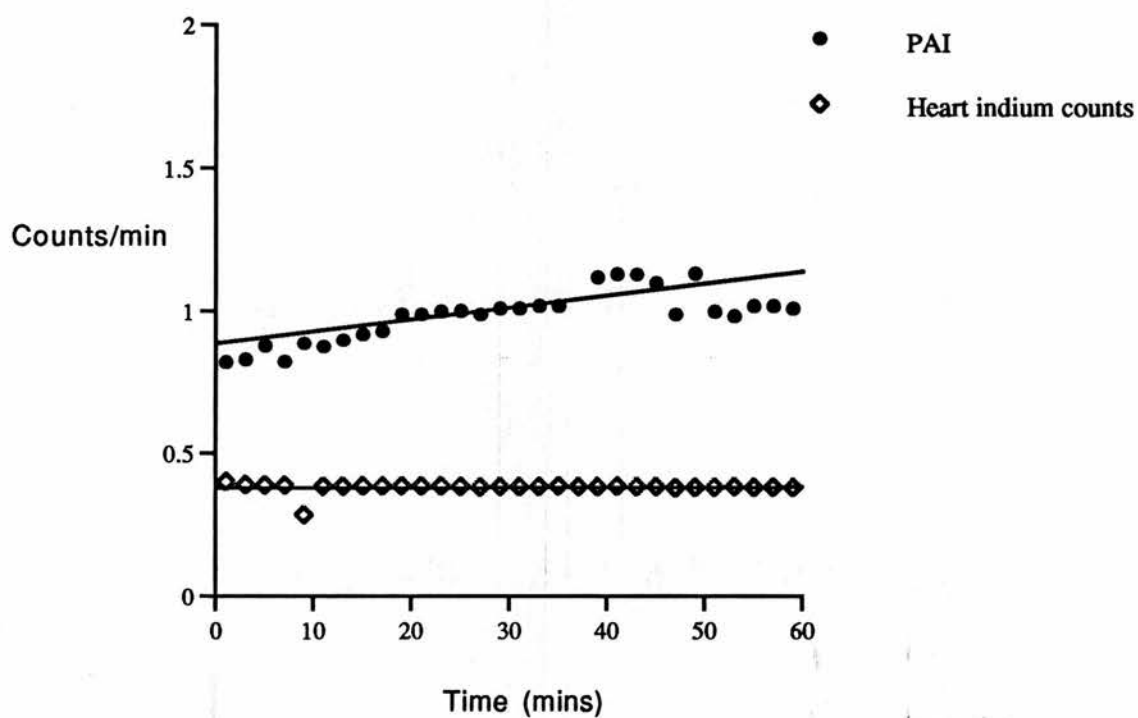


Figure 5.8 Graphs recorded from post-oesophagogastratomy patient number 20. The PAI= $1.90 \times 10^{-3}/\text{min}$ and the slope of the heart curve is $-0.5 \times 10^{-3}/\text{min}$.

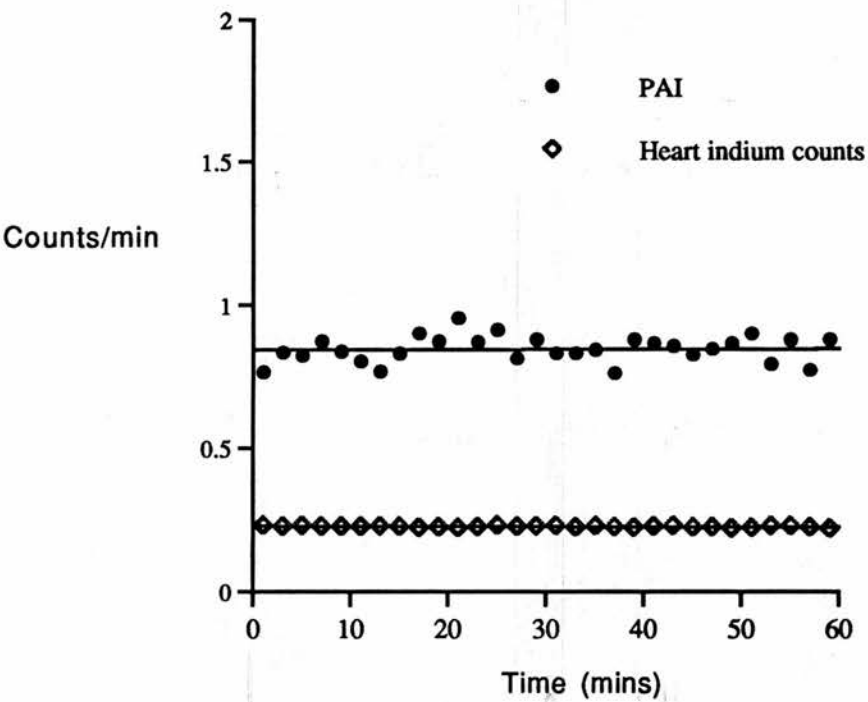


Figure 5.9 Graphs recorded from healthy volunteer number 1. The PAI = $0.06 \times 10^{-3}/\text{min}$ and the slope of the heart indium curve is $-0.91 \times 10^{-3}/\text{min}$.

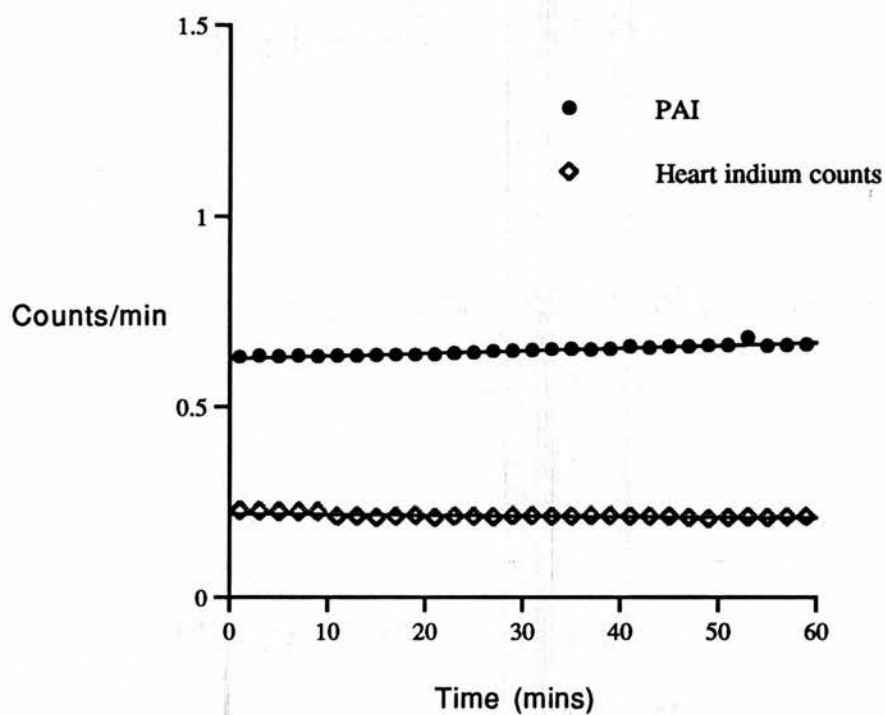


Figure 5.10 Graphs of recordings from healthy volunteer number 3. The PAI = $0.6 \times 10^{-3}/\text{min}$ and the slope of the heart indium curve is $-0.42 \times 10^{-3}/\text{min}$.

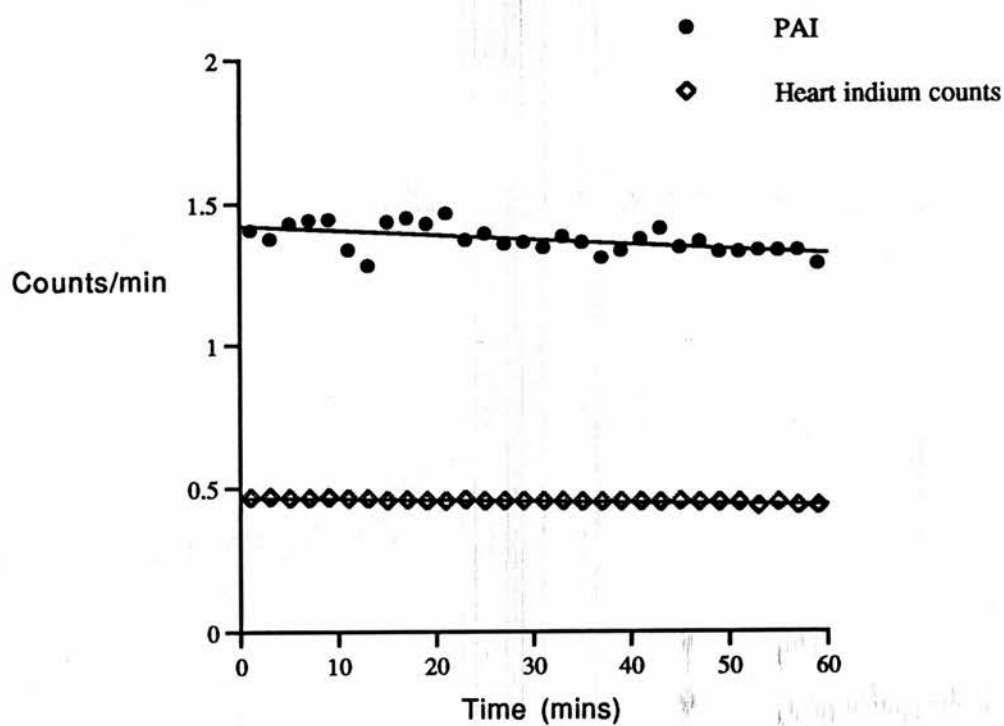


Figure 5.11 Graph of recording from patient number 1 with ARDS. The PAI= $-0.7 \times 10^{-3}/\text{min}$ and the heart indium trace displays a slope of $-0.5 \times 10^{-3}/\text{min}$.

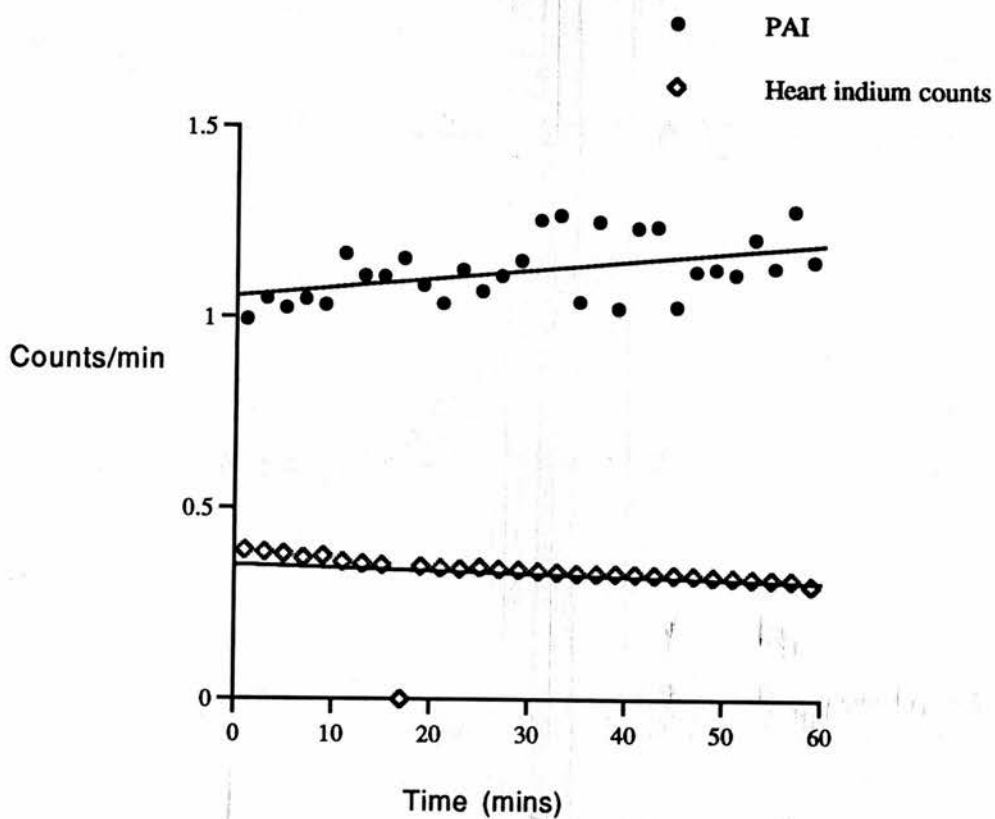


Figure 5.12 Graph of recordings from patient number 6 with ARDS. The PAI = $1.45 \times 10^{-3}/\text{min}$ and the slope of the heart indium curve is $-3.0 \times 10^{-3}/\text{min}$.

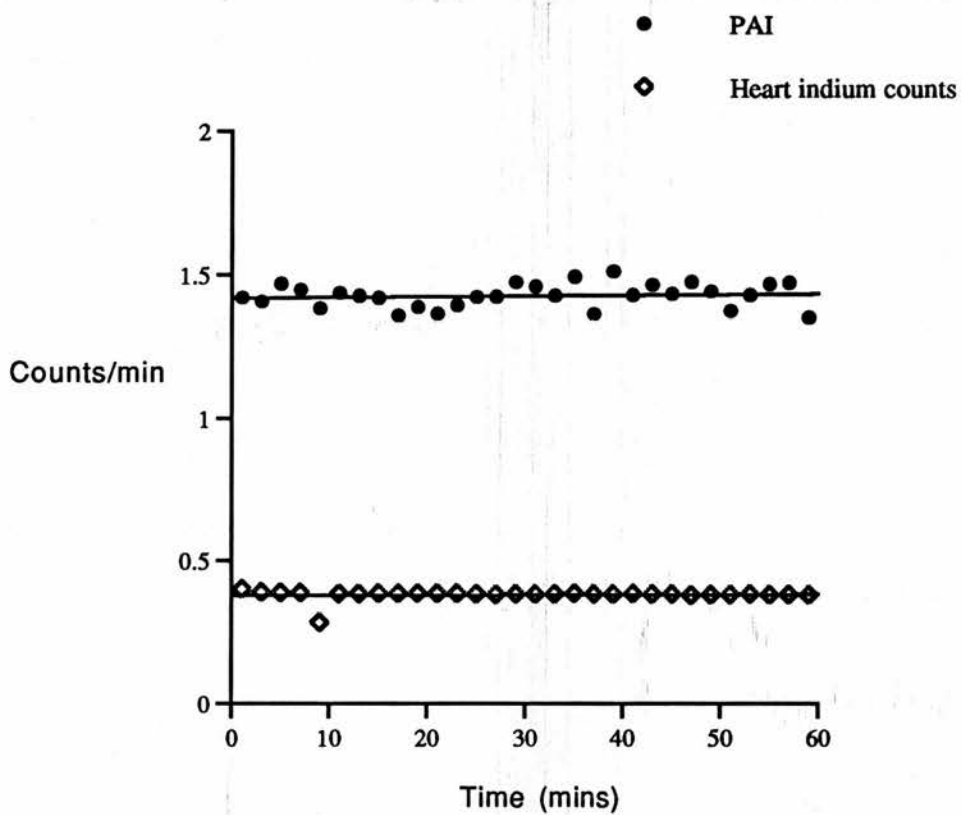


Figure 5.13 Graph of recordings from patient number 9 with ARDS. The PAI = $-0.8 \times 10^{-3}/\text{min}$ and the slope of the heart indium is $-1.04 \times 10^{-3}/\text{min}$.

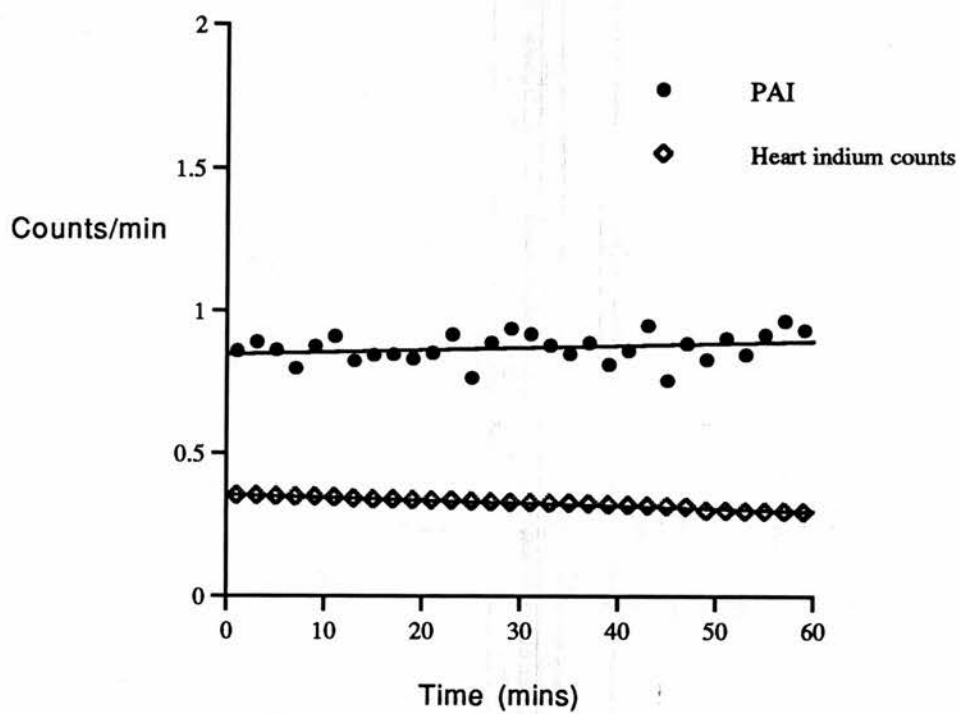


Figure 5.14 Graph of recordings from patient number 12 with ARDS. The PAI = $1.0 \times 10^{-3}/\text{min}$ and the slope of the heart indium is $-2.9 \times 10^{-3}/\text{min}$.

CHAPTER SIX SUMMARY

Despite improvements in the delivery of critical and intensive care only marginal impact has been made on the mortality and morbidity associated with ARDS and as yet, no pharmacological intervention has been demonstrated to have a substantial impact on the disease process. In part, this is likely to reflect the fact that using current diagnostic criteria, extensive and catastrophic lung damage has occurred by the time the physician can confidently diagnose the patient as suffering from ARDS. The lung is then so severely injured as to render the process of repair and remodelling an overwhelming task. From this perspective, we may reasonably consider that further improvements in the outlook for patients with ARDS are most likely to come from the earlier recognition of evolving acute inflammatory response.

Studies performed in Edinburgh, on the bronchoalveolar lavage and blood from patients suffering known precipitants of lung injury but who are within the earliest stages of the risk period, have demonstrated that it is potentially possible to identify those patients who are at highest risk of proceeding to established lung injury syndromes. However, bronchoalveolar lavage may be difficult to perform early in the at-risk period and the preparation and processing of the samples including the performance of enzyme-linked and radioimmunoassays involved may take hours to days before the results may be available.

The application of measurements of pulmonary microvascular permeability are attractive for several reasons. Pathological studies suggest that the earliest events in

the pathogenesis of ARDS occur within the pulmonary microvasculature with a resultant breakdown in the alveolar-capillary barrier function. Furthermore, enhanced pulmonary microvascular permeability is central to our understanding of the pathophysiology of established ARDS and was recognised by the American-European Consensus statement as an important area in which to direct further research.

In this thesis I have evaluated the use of a fully portable dual system which utilises miniature scintillation counters and provides a non-invasive method of pulmonary microvascular permeability based on charting the transit of radiolabelled proteins. I have shown from phantom experiments that the system is capable of recording from both indium and technetium radioisotopes simultaneously in the setting of the Intensive Therapy Unit but that the miniature scintillation counters are likely to sample only a limited volume of lung tissue in any given patient. This is an important finding as the principle of the technique depends on the extrapolation of data obtained by recording over a small area of lung tissue to make an inference on the function of the lung as a whole. Whilst this may be applicable in disease processes which are homogeneously distributed throughout the lung, we are now aware that ARDS is heterogeneous in nature.

More encouraging results were observed when I studied patients earlier in the disease process. In patients in the immediate post-operative phase following oesophageal resection, I was able to demonstrate that the protein accumulation index obtained

correlated with both the $\text{PaO}_2/\text{FiO}_2$ ratio and the circulating plasma elastase. This work supports the role of the neutrophil as a key effector cell in the earliest stages of acute inflammatory lung injury and is consistent with the contention that the first steps in the initiation of acute inflammatory lung injury may occur within the pulmonary intravascular space. I was, however, unable to demonstrate that the occurrence of microvascular permeability is reflected by the circulating level of several currently available endothelial markers although the development of circulating markers of endothelial damage which are specific to the pulmonary or systemic circulation should prompt further studies in this area. Circulating markers which reflect damage to the epithelial barrier such as surfactant proteins or other products of the alveolar epithelial cells may provide more appropriate markers

An important observation in this thesis is the number of negative values recorded for PAI in the post-oesophagogastrectomy group. The possible explanations for this are discussed in chapter 5 but is most likely to reflect the accumulation of indium-transferrin which is probably associated with the oesophagogastric anastomosis and is sampled by the heart probe. This explanation is supported by the frequent finding of positive heart indium slopes in these patients which was not observed in either the healthy volunteers or patients with ARDS (figures 5.5 - 5.14). Although, in retrospect, this would seem a likely consequence of this technique it was unexpected as the same principle had been employed in a similar patient group (Rocker et al. 1988 b) and in patients who had undergone cardiopulmonary bypass with median sternotomy (MacNaughton et al. 1992) This may reflect the fact that the Nottingham

group used a larger focused collimator and the London group redesigned the collimation on the mini-scintillation detector (Hunter DN et al. 1990). Should these studies be repeated it would be extremely valuable to adopt the protocol employed by the Netherlands group in which serial venous sampling was used throughout the study period to determine the activity of the circulating radionuclides and compare these results to those obtained by the heart probe (Raijmakers et al. 1993).

Although the power of these studies is limited by the patient numbers, it seems unlikely that the detection of elevated pulmonary endothelial permeability *per se* will provide a reliable predictive indicator to the occurrence of impending acute lung injury. Perhaps such hopes are unrealistic when one considers that it is the pulmonary epithelium which represents the rate limiting step preventing alveolar flooding and as such, increased endothelial permeability may depict a *forme fruste* which may resolve without further consequence to the patient. Furthermore, both ALI and ARDS, as currently defined, encompass a number of disparate conditions which may evolve through different pathways. Thus a combination of indices with predictive value should be tested to identify algorithms which may be applicable to a broader patient groupings.

Finally, it is important to discuss whether the measurement of pulmonary microvascular permeability is likely to be beneficial in the management of patients with established ALI and ARDS. Currently the management of these patients is largely supportive and does not depend on knowing, if and to what extent

permeability is abnormal, it may be argued that no such measurement is necessary and this is perhaps one reason that such techniques have not been widely applied. It has been suggested that the degree of permeability reflects the severity of the underlying lung injury and as such may have some bearing on prognosis (Velazquez et al. 1991, Sinclair et al. 1994). If further studies can repeat these observations then useful data relating to patient outcome might be expected. Furthermore, as new therapies and ventilation strategies are applied in the setting of controlled clinical trials it may be reassuring to know that they do not lead to an increase in permeability.

Whilst this technique is unlikely to be useful within the setting of established ARDS, the benefits of a reliable should be undisputed. As further mediators are described within the inflamed and damaged lung it will be of important to recognise and identify those which most reliably reflect the underlying pathophysiological response. Furthermore, as we move to evaluate new therapeutic agents and modalities, within the setting of controlled clinical trials, it may prove useful to determine whether any intervention leads to a worsening of pulmonary permeability.

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